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CHANGES IN THE ELASTICITY OF THE AORTA WITH AGE

JOSEPH KRAFKA JR., M.D.

AUGUSTA, GA.

A series of investigations on the elasticity of the aorta has definitely established the curve of extensibility to be an exponential curve with the hollow at the 50-100 Gm. tension level (Krafka [1b]). This is in keeping with the observations of Yater and Birkeland,[2] who noted that the greatest proportional stretch for strips was at the 50-100 Gm. level. The same general phenomenon may also be seen in the curve showing the relation of volume to pressure as given by Wiggers.[3]

That there is a shift in the elasticity of the aorta with age is a generally accepted fact. Some investigators have claimed that this is not a progressive phenomenon, but that there is an increase in distensibility from birth to the twentieth year with a gradual decline from that time to old age. It is of interest to note that of 100 specimens studied by Yater and Birkeland, only 2 were from the 0-20 year age group; hence the conclusion is hardly justified. In any event, the increase in extensibility remains unexplained.

Loss in elasticity from maturity to senescence is considered to have an etiologic factor in (1) fibrosis, (2) loss in muscle tonus and (3) degeneration of elastic fibers. Since three separate histologic elements are involved, both quantitatively and qualitatively, the problem becomes complex in its variety of possible combinations leading to the same end result. An estimation of the relative values of each factor is possible only after a careful analysis of the elongation curve into the separate roles of muscle, elastic tissue and collagenous fibers.

Analysis of the elongation curve by the method of comparative elastic moduli indicates that the hollow portion of the curve is the region

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From the University of Georgia School of Medicine.

This series of investigations was made possible by grant 469 of the Committee on Scientific Research of the American Medical Association.

1. Krafka, J., Jr.: (*a*) Arch. Path. **23**:110, 1937; (*b*) Am. J. Physiol. **125**: 1, 1939.

2. Yater, W. M., and Birkeland, J. W.: Am. Heart J. **5**:781, 1930.

3. Wiggers, C. J.: Am. J. Physiol. **123**:644, 1938.

of action of muscle and elastic fibers, while the straight line portion is due to the higher moduli of the collagenous fibers (Krafka [1b]). Furthermore, normal diastolic and systolic pressure equivalents fall on the hollow of the curve (Krafka [4]). With these points in mind, it is

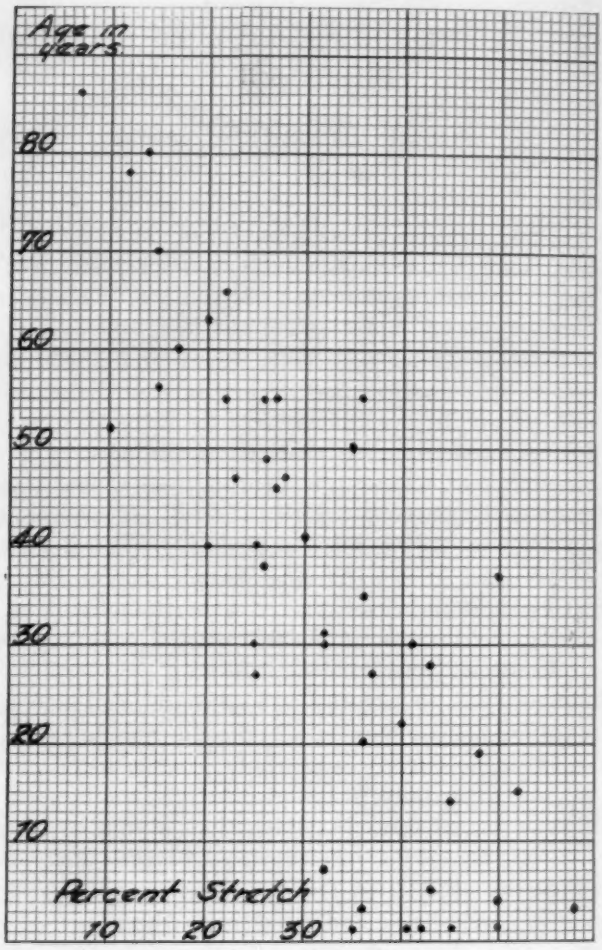

Chart 1.—Scatter diagram showing percental stretch of aortic strips for the 100 Gm. tension level. Note the marked decrease with age.

possible to evaluate the relative roles of the three histologic elements in the loss of elasticity of the aorta with age.

4. Krafka, J., Jr.: Am. J. Physiol., to be published.

METHODS

Elongation curves for longitudinal strips of aortas secured at autopsies were made on the recording serigraph as described in a

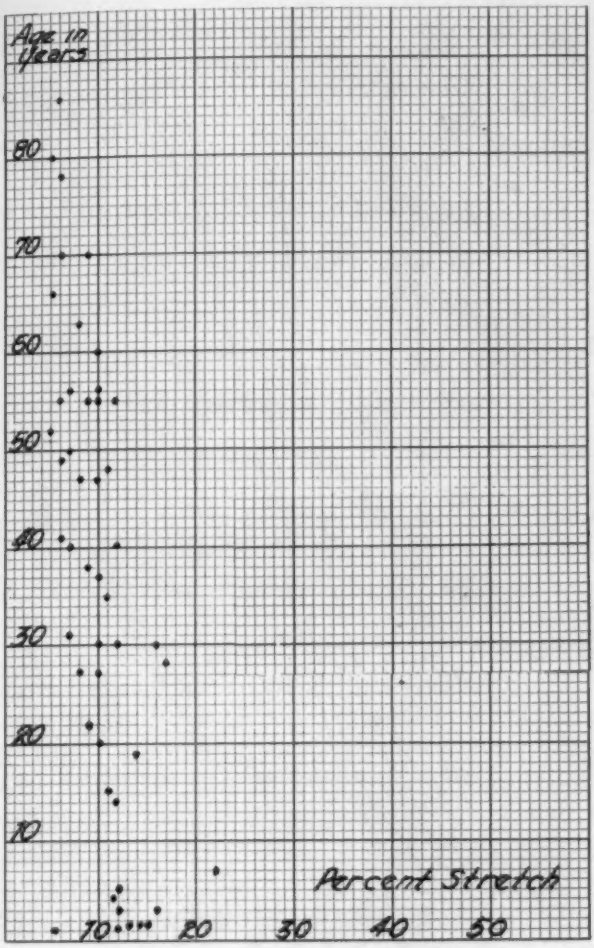

Chart 2.—Scatter diagram showing percental stretch of aortic strips for the 200-100 Gm. tension level. Note the gradual decrease with age.

previous paper (Krafka [1b]). From these curves, percental extensibility for 100 Gm. tension was measured, and the data recorded as a scattergram (chart 1). Measurements were next made for the additional extension at from 100 to 200 Gm. tension, i. e., for the straight line

portion of the curve. These values are presented in a second scattergram (chart 2).

From the scattergrams several facts are at once apparent. 1. There is no increase in distensibility from birth up to the twentieth year.

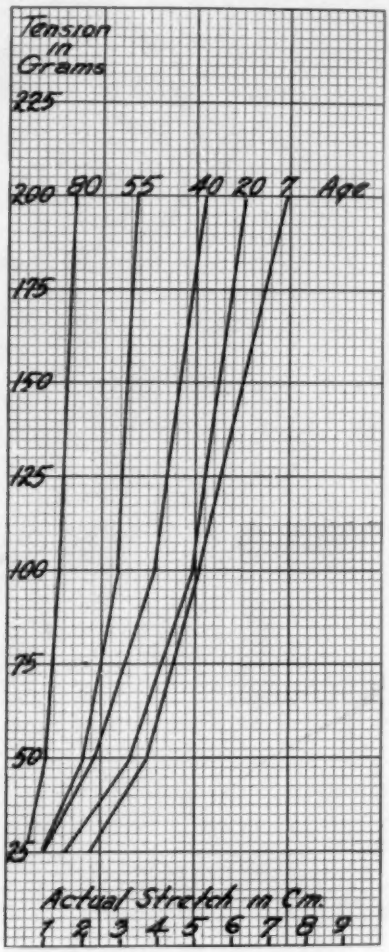

Chart 3.—Serigrams of standard strips of human aortas 10 cm. long and 5 mm. wide. Note the loss of elasticity with age.

2. There is a gradual loss in elasticity with age throughout life in (*a*) the hollow portion of the curve and (*b*) the straight line portion of the curve. 3. The difference, however, is not as marked for the straight line portion of the curve as it is for the hollow portion of the curve.

The same phenomena are apparent from a comparison of the elongation curves for the various ages as given in chart 3, and from the data in the table.

Relationship of Percental Stretch of 10 Cm. Strips of Human Aorta at Various Ages in Man (New Series)

Age, Yr.	Thickness, Mm.	Percental Stretch at Given Number of Grams				Prevailing Blood Pressure
		50	100	200	200-100	
7	1.8	39	51	75	24	.....
20	2.6	35	51	64	13	.....
31	2.6	20	32	42	10	275/140
37	2.9	23	36	47	11	140/ 90
37	1.76	35	50	60	10	290/145
40	2.88*	20	40	53	13	195/130
41	3.18	22	30	35	5	105/ 75
47	2.34	18	27	35	8	230/160
48	2.46	20	28	39	11	145/ 83
55	2.90*	20	28	35	7	200/120
55	2.92*	24	36	48	12	175/ 95
80	2.36*	10	14	18	4	180/110

* The aorta was markedly sclerotic.

COMMENT

A general loss in the elasticity of the aorta has frequently been interpreted as due to an increase in the number of collagenous fibers. This point is significant in my theory of intimal herniation as the principal mechanical factor in the production of sclerosis (Krafka [1a]). This concept, however, requires some alteration, since it is at once evident that a progressive loss of elasticity in the upper portion of the curve should not occur unless there is (1) a definite change in the elastic properties of the collagenous fibers or (2) a change in the architecture of the wall. A mere increase in the number of fibers in itself would fail to produce the aging effect.

A significant observation of Yater and Birkeland has a bearing on this point. Although they did not record their data, they concluded that there is no direct correlation between the thickness of the wall with age and loss in elasticity. The same fact was noted in the present series. For example, at the age of 80 years the thickness of the wall was 2.36 mm.; at 55 years, 2.92 mm.; at 41 years, 3.18 mm. Comparison of the elastic moduli shows that loss in elasticity is not primarily due to an increase in white fibers.

Another consideration enters the problem. Hallock and Benson [5] reported that the volume of segments of the aorta under pressure of

5. Hallock, P., and Benson, I. C.: J. Clin. Investigation **16**:595, 1937.

100 mm. of mercury increases with increasing age. Thus the volume per centimeter of length at 100 mm. pressure at the age of 20 years is 2.40; at 50 years, 2.60; at 78 years, 3.20. This relationship may be accounted for on the simple assumption of a relaxation of the tube due to the failure of the musculoelastic tissue to maintain its tonus, which throws the entire recoil resistance on the white fibers. This in itself may produce an actual thinning and at the same time increase the modulus of elasticity. Hallock and Benson [4] considered the increase in volume to be due to an actual breakdown and disappearance of the elastic fibers, as established by Zon, but sections of aorta at old age still show an appreciable amount of elastic fibers.

Thinning of the aortic wall has been previously postulated on theoretic grounds (Krafka [1a]) to be an adaptive mechanism which, by changing the relationship of the thickness of the wall to the diameter of the vessel, attempts to reestablish the original elasticity.

The key to the analysis of the problem was incidentally secured from tests of aortic strips before and after putrefaction (Krafka [1b]). The immediate effect of putrefaction is to decrease the elastic modulus. But when calculations are made by the incremental method that bears directly on the collagenous fibers, the distended strips show the moduli of white fibers. In these tests an intact strip of aorta, 10 cm. long, was tested and after putrefaction for twenty-four days, the same strip was fixed in the clamps of the serigraph at the points of the initial test. When the strips were fastened in the clamps with an initial state of no tension, instead of relaxed tension, a typical straight line curve was obtained.

Anomalous as it seems, loss of tone by relaxation of muscle elastic tissue thus increases the elastic modulus and at the same time increases the volume.

As evidence that the serigraph method is adaptable to this problem, calculations of the percental stretch of the wall based on the pressure-volume relationships of Hallock and Benson [4] give values which are closely comparable to those for the stretch observed with equivalent tensions on the serigram. Thus for the age of 20 years the calculated stretch for 200 mm. is 32.6 per cent, while the measured stretch on the serigram is 39 per cent. For 78 years the total stretch calculated is 11.2 per cent; for 80 years on the serigram it is 5 per cent.

These relationships warrant the conclusion of Hallock and Benson [4] that in old age the aorta assumes the role of a capacity chamber for the reception of cardiac output without due strain.

If a comparison is now made between the respective stretches for the ages 20 and 80 years, it will be seen that practically the entire loss of elasticity of the aorta may be accounted for on the basis of a relaxation

of the musculoelastic elements effective in the hollow portion of the curve. Thus the total stretch at 20 years is 64 per cent and that at 80 years is 18 per cent, with a difference of 46 per cent. This is a close approximation of the stretch at 20 years at 100 Gm., namely, 51 per cent (table).

A comparison of the 200-100 Gm. ranges for the ages 20 years and 80 years gives the values 13 per cent and 4 per cent, respectively. Hence the difference, 9 per cent, may be taken as the loss in elasticity due to a fibrosis factor. This compared with the 46 per cent would mean an approximate ratio of 1 to 5. Or stated as a generality, 80 per cent of the loss in elasticity of the aorta with age is due to failure of the musculoelastic system; 20 per cent, to fibrosis.

In conclusion, the statement may again be emphasized that sclerosis and loss in elasticity are not entirely correlated. The aorta may lose its characteristic as a rebound tissue without evident sclerosis, and localized sclerosis may exist without evident loss in elasticity.

A question is frequently asked as to the therapeutic application of the study of elasticity. The answer is that the present practice of rest in aortic dysfunction is entirely rational. As to medication designed to reestablish elasticity of the aorta, a search should be made for a smooth muscle tonic which at the same time would not affect the tonus of the heart.

CONCLUSIONS

1. Loss in elasticity of the aorta with age results from two factors: (*a*) distensibility, 80 per cent, and (*b*) fibrosis, 20 per cent.

2. Increase in distensibility from birth to maturity is not established.

3. No direct correlation exists between elasticity, blood pressure, sclerosis and thickness of the aortic wall.

SIMMONDS' DISEASE (PITUITARY CACHEXIA) IN AN AGED MAN WITH DEMENTIA PRAECOX

MYRTELLE M. CANAVAN, M.D.
BOSTON

In an autopsy on a man 72 years of age, who had died about three months after fracturing a femur, it was noted that the subject was greatly emaciated. The skin literally just covered his bones, and while his weight had not been recorded since the injury, he had weighed just previously to the injury 138 pounds (62.5 Kg.). The dead body would not have registered more than 90 pounds (41 Kg.), and his death had been expected every day for six weeks from sheer weakness, though he had taken food fairly regularly.

The patient suffered from dementia praecox and had been shifted from one mental disease hospital to a second and then to a third, where he had spent the last twenty-eight years being occupied, when able, in light ward work. In July 1932 he was put to bed because of edema of his legs, and this subsided. On August 1 he had in some way fractured his left femur and remained in bed, his color, strength and weight decreasing until October 20, when he died.

The body measured 178 cm. in length; there was little hair on the head, in the axillas or over the pubis. This would agree with Berman's[1] idea of pituitary inactivity, since he stated that the removal of the anterior lobe of the pituitary results in loss of hair.[2]

When the pituitary was removed and held by a dural tag in forceps, it resembled an edematous string.

The chronic lesions found were: hypertrophy of toe nails, enlarged mesenteric lymph nodes, diverticula of the jejunum, a few plaques in the coronary arteries, aorta and basal vessels, a small, flabby heart, a large gallbladder, a *very small stomach* (not much larger than the gallbladder), hypertrophy of the prostate, atrophy of the testicles and thickening of the pia-arachnoid. The acute changes were: pressure sores on one ankle, fluid in the pleural cavities and bronchopneumonia.

From the Pathological Laboratory of the Massachusetts Department of Mental Health.

1. Berman, L.: The Glands Regulating Personality, ed. 2, New York, The Macmillan Company, 1928, chap. 8.

2. I have observed that pubic hair shows marked differences in age groups: between puberty and 25 years this hair is often glistening, curly and abundant. After 40 the hair is dryer and more nearly straight, streaks of gray increasing with age, until at 90 it is represented by a few thin spears of white hair. This loss is more marked in men than in women.

That he had only mild thickening of the blood vessels and no tuberculosis or malignant growth to account for the emaciation was of interest; therefore the association of this loss of weight with the edematous small pituitary was unavoidable. Correspondence with the superintendents of Massachusetts state hospitals brought no reports of any clinical manifestations of pituitary lack in their patients.

In an active pathologic service with the Massachusetts Department of Mental Health for over twenty years I have never seen a pituitary

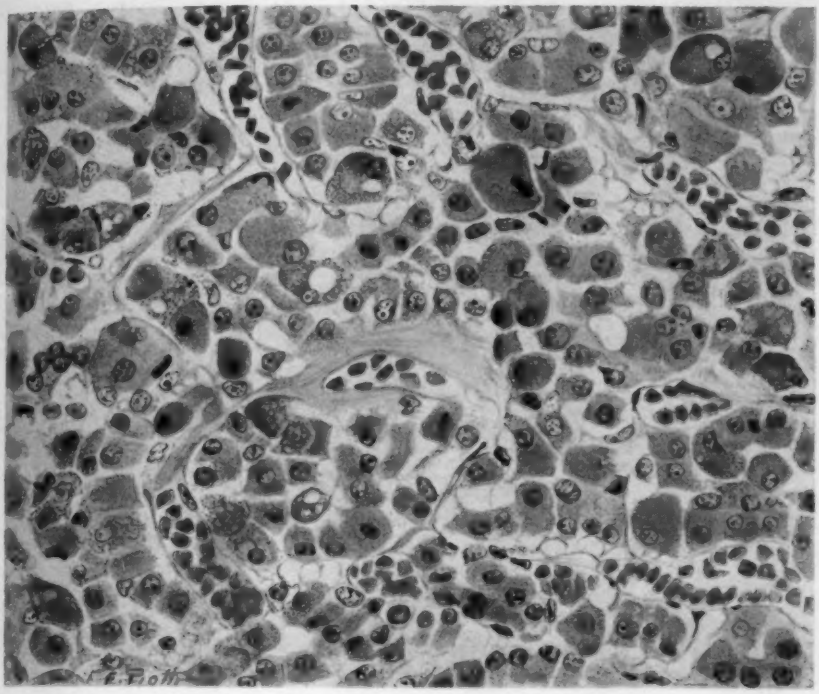

Fig. 1.—Normal pituitary. Note numerous sinusoids filled with red blood cells. There is a suggestion of acinar arrangement. The abundant acidophils vary in size and in depth of protoplasmic coloring. The chromophobes are with or without protoplasm. The basophilic cells are large and finely granular, with nuclei often eccentric, and show the characteristic vacuole near the nucleus. Hematoxylin and eosin; × 540.

of similar gross appearance. Microscopically, the anterior lobe consisted chiefly of collapsed and dull polychromatic edematous cells and showed many free nuclei and shadows of cell outlines. The acidophils showed marked vacuolation of the cytoplasm; in 50 fields an average of 2 solidly granular acidophils was found. There was definite paucity

of basophils. The chromophobes appeared normal. There was no increase of interstitial tissue, and the walls of the sinusoids were nearly in apposition.

Silver,[3] in a review of the literature to 1933, stated that persons suffering from Simmonds' disease show certain characteristic clinical and pathologic features: Their ages vary from 9 to 69. Their disease occurs after pregnancy, infection, accidental injury, alcoholism or an unknown cause, and the duration varies from seven months to

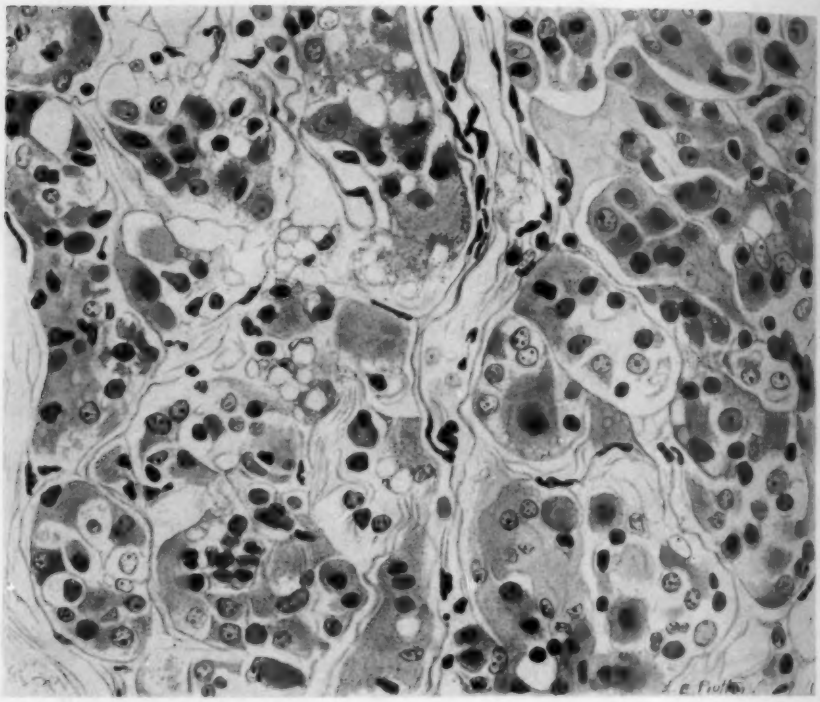

Fig. 2.—Pituitary of a patient with Simmonds' disease. The collapsed sinusoids contain no red blood cells, and there is proliferation of their endothelial lining. There are no basophils. The majority of the cells are vacuolated acidophils. Hematoxylin and eosin; × 540.

forty-four years. They show premature aging, loss of weight, strength, libido, teeth, pubic and axillary hair, and atrophy of the genitalia and of the jaw. Besides these signs, hypotension, hypothermia, eosinophilia and anemia are found.

The patient under consideration was 72; his signs came on after fracture of a femur, and their duration was eleven weeks. He pre-

3. Silver, S.: Arch. Int. Med. **51**:175, 1933.

sented some of those changes given in Silver's review, namely, cachexia, marked asthenia, loss of pubic and axillary hair and atrophy of the testicles, and he was very pale. His advanced age, in contrast to those mentioned by Silver, makes the clinical signs, with the exception of the marked loss of weight, less significant.

The pathologic changes (condensed from Silver's review) are smallness of viscera and atrophy of the endocrine glands in some cases. Changes in the pituitary are prominent. These include loss of weight of the gland and smallness of the gland, with fibrous, cystic, tuberculous or hemorrhagic lesions in the anterior lobe. The patient concerned in this report showed the small stomach, the atrophy of the testicles and the marked degeneration in the anterior lobe of the unweighed gland.

SUMMARY

The patient was a 72 year old man with dementia praecox. Marked emaciation occurred after a fracture of the femur. The duration of Simmonds' disease was eleven weeks. Grossly, the pituitary was small and edematous. Microscopically, collapse of the sinusoids was seen as well as varying degrees of degeneration of the cells in the anterior lobe, particularly of the acidophils.

PRIMARY AMYLOID DISEASE OF THE MYOCARDIUM AND BLOOD VESSELS

REPORT OF A CASE WITH DEATH FROM MYOCARDIAL FAILURE

CHAPMAN H. BINFORD, M.D.
Passed Assistant Surgeon, United States Public Health Service
DETROIT

The literature records few cases in which death was due to cardiac failure brought about by deposition of amyloid in the myocardium. Kerwin [1] collected 5 cases and reported 2 additional cases of his own. He stated that Wilks,[2] in 1856, recorded the first case. Budd [3] reported a case, that of a 75 year old man who died of carcinoma of the prostate; the heart showed advanced myocardial amyloidosis. Larsen,[4] in addition to a case observed by himself, in which there was definite myocardial failure (included in Kerwin's review), mentioned a case observed by Beneke and Bönning,[5] that of a man aged 70 years who entered the hospital in a moribund condition with a diagnosis of bronchitis; extensive amyloid deposition was found in the heart, venae cavae and lungs.

Bannick and his co-workers [6] reported diffuse amyloidosis occurring in a 44 year old man who had died of a combination of profound renal, adrenal and hepatic insufficiency. In addition to large amounts of atypically staining amyloid deposited in these organs and in the spleen, the walls of many blood vessels showed acellular hyaline material which was stained by congo red. The myocardial muscle cells revealed marked atrophy, and the stroma was increased because of narrow bands of hyaline substance, which also had an affinity for congo red. In some areas muscle fibers had disappeared entirely.

Primary systemic amyloidosis is a rare entity. In their recent review Koletsky and Stecher [7] found 22 recorded cases and added a case.

In the case to be reported, cardiac amyloidosis appeared to have been responsible for myocardial failure and death. As there was no other associated chronic disease, the condition must be classed as primary.

From the Laboratory of the United States Marine Hospital.

1. Kerwin, A. J.: J. Lab. & Clin. Med. **22**:255, 1936.
2. Wilks, S.: Guy's Hosp. Rep. **2**:105, 1856.
3. Budd, J. W.: Am. J. Path. **10**:299, 1934.
4. Larsen, R. M.: Am. J. Path. **6**:147, 1930.
5. Beneke, R., and Bönning, F.: Beitr. z. path. Anat. u. z. allg. Path. **44**: 362, 1908.
6. Bannick, E. G.; Berkman, J. M., and Beaver, D. C.: Arch. Int. Med. **51**: 978, 1933.
7. Koletsky, S., and Stecher, R. M.: Arch. Path. **27**:267, 1939.

REPORT OF CASE

M. T. C., a white man aged 56, married, a veteran of the Spanish-American War, was admitted to the hospital Aug. 2, 1938. He had not worked for several years but formerly was employed as a meat cutter in a grocery store. With the exception that his mother died of tuberculosis, his family history was not significant. His personal history revealed that he had had an attack of acute bronchitis twenty years ago. He used alcohol in moderation and until five weeks before admission had used tobacco. He stated that his health had been fairly good until about eight months before admission, when he became dyspneic on exertion. Symptoms had become progressively worse. About one month before admission he had spent ten days in another hospital, where a thorough study was made, and the attacks of dyspnea were thought to be due to bronchial asthma. However, based on electrocardiographic studies, an additional diagnosis of anterior myocardial infarction (old) was made. At the same time he was treated for mycosis of the finger nails and toe nails. During the month prior to admission here there had been a productive cough, orthopnea and swelling of the ankles.

Physical Examination.—The patient was a fairly well developed middle-aged man, weak, somewhat cyanotic and apparently acutely ill. There were crepitant and coarse rales throughout the chest and suppression of breath sounds over the base of the right lung. The heart was not enlarged to percussion, the rhythm appeared to be tic-tac and no murmurs were heard. The blood pressure was 92 systolic and 78 diastolic. The peripheral vessels were thickened and tortuous. No peripheral edema was present. The temperature was 37 C. and the pulse rate 100. The urine showed a faint trace of albumin, and the specific gravity was 1.026. The Kahn test of the blood was negative. The erythrocytes numbered 4,550,000, and the leukocytes 9,000 per cubic millimeter. The hemoglobin content was 75 per cent. A differential count revealed 85 per cent neutrophils, 11 per cent lymphocytes and 4 per cent monocytes. Roentgen examination of the chest showed bilateral irregularity of the diaphragm due to pleural adhesions, thickening of the pleura of the right lower region of the chest, thought to be associated with some pleural effusion, and compression atelectasis of both lower pulmonary fields. The heart was not enlarged. An electrocardiogram exhibited extremely low voltage throughout.

Course.—Under a regimen of rest in bed, digitalis and sedation the patient temporarily became more comfortable. It was soon the opinion of his physicians that the dyspnea was due to myocardial degeneration and not to bronchial asthma. About one month after admission he was allowed to be up for short periods. Later, however, his dyspnea and nocturnal wheezing returned, his ankles showed edema, and a roentgen picture of the chest revealed bilateral hydrothorax. Repeated thoracenteses were done on alternate sides, with recovery of approximately 1,000 to 2,000 cc. of clear fluid of a specific gravity of about 1.012. The patient complained persistently of flatulence and vague precordial distress. Mercurial diuretics and ammonium chloride were also administered from time to time. The blood pressure taken three months after admission was 80 systolic and 60 diastolic. He became progressively weaker and died March 10, 1939, about seven months after his final hospitalization and fifteen months after the onset of severe symptoms.

Autopsy.—The autopsy began two and one-half hours post mortem.

Externally the body presented no abnormalities with the exception of scaliness and partial loss of several finger nails. No lesions of the skin were seen.

There was a small amount of clear fluid in the abdominal fossae. The pleural cavities contained much fluid; that on the left was clear and yellowish, but that on the right was turbid and dark red. Both cavities showed many adhesions between the visceral and the parietal layer of pleura.

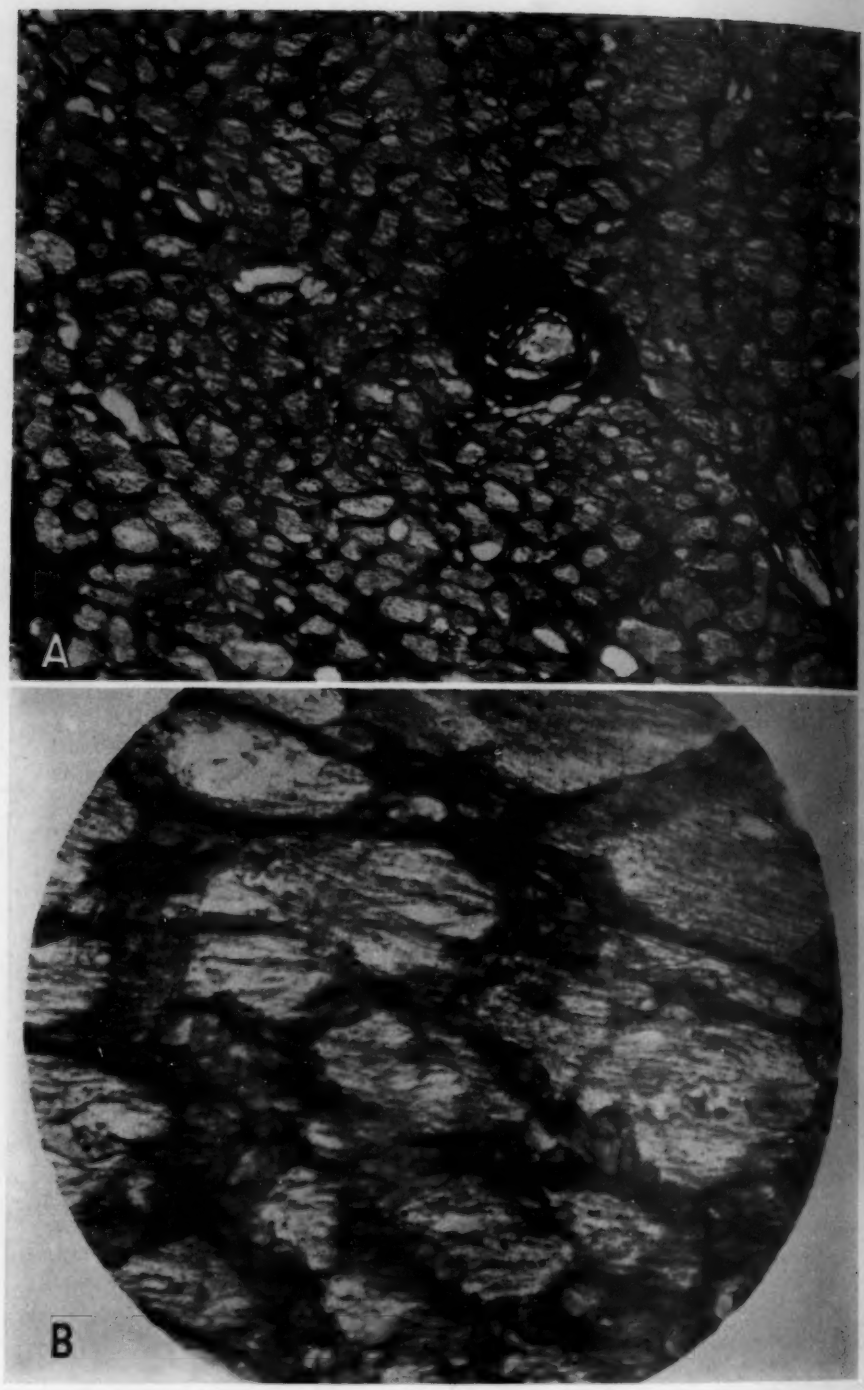

Fig. 1.—*A*, interstitial infiltration by hyaline material and deposition of amyloid in an arteriolar wall in the left ventricle of the heart; fast green (Masson's method); × 150. *B*, higher magnification of *A*; × 600.

The heart, which weighed 400 Gm., was normally situated in the thorax. The greatest transverse diameter measured 14 cm. There were only a few cubic centimeters of pericardial fluid. The pericardium appeared normal. The right ventricle was slightly dilated and the left contracted. On section the myocardium of the right and left ventricles and interventricular septum was firm and its color pinkish red. The wall of the left ventricle measured 10 mm. and that of

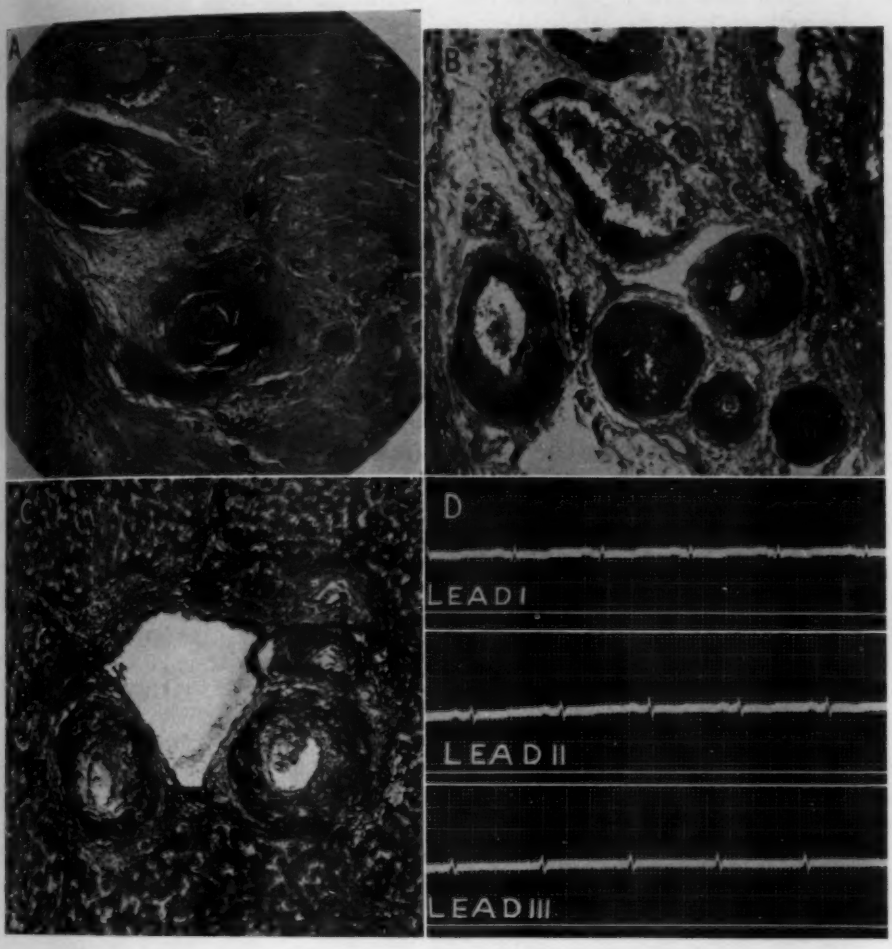

Fig. 2.—*A*, obliterative deposition of amyloid in the walls of small vessels in the left ventricle of the heart; gentian violet; × 150. *B*, amyloid deposits in periadrenal arterioles and venules; methyl violet; × 150. *C*, amyloid deposits in thick-walled interlobular vessels of the liver; methyl violet; × 90. *D*, electrocardiogram made Sept. 6, 1938.

the right ventricle 2 to 4 mm. in thickness. The leaflets and rings of the various valves appeared normal. The coronary arteries were patent and presented no significant sclerotic changes. In the right auricular appendage were numerous soft friable thrombi.

The abdominal aorta exhibited a few intimal atheromatous plaques. Changes in other levels were minimal. The left common iliac artery was plugged by a friable soft gray clot, but the extremity showed no evidence of infarction. There was occlusion of the branch of the pulmonary artery supplying the lower lobe of the right lung, with resulting massive infarction. A small infarct was also present in the inferior border of the upper lobe of the right lung. The liver, which weighed 1,000 Gm., showed centrolobular congestion. The right kidney presented several small infarcts. There was a single small infarct in the spleen.

The other organs presented no abnormalities. While the autopsy explained the death on the basis of massive pulmonary infarction, there was no anatomic observation to explain the cardiac failure.

Microscopic Examination.—Sections of the left ventricle of the heart showed significant amounts of opaque amorphous substance deposited in the epicardium and myocardium. It was most conspicuous in the media and perivascular zones of the small blood vessels but also was seen as focal aggregates replacing muscle fibers or as pericapillary thickenings diffusely spread between individual fibers. The muscle fibers often appeared fragmented, as if being constricted or choked by the infiltrating substance. The media of the numerous arterioles and venules infiltrated by the substance showed much thickening and loss of recognizable muscle fibers. The lumens of the smaller vessels were markedly stenosed. The vascular endothelium was preserved.

The substance was stained bright yellow by Van Gieson's solution of trinitrophenol and fuchsin, pale blue by Romanowsky's stain,[8] pink by hematoxylin and eosin (frozen section), pink by congo red and pale to dark green by fast green used instead of light green in a modification [8a] of Masson's trichrome method. With dilute iodine on frozen sections, the thicker deposits became pale brown, and after exposure to 5 per cent sulfuric acid the color changed to greenish black. All stains were made on material fixed in solution of formaldehyde, U. S. P., and with the exception of those on frozen sections were on paraffin-embedded material. With methyl violet and gentian violet the deposits in the walls of blood vessels stained the characteristic reddish violet of amyloid. The fine deposits about the individual fibers occasionally showed metachromatic staining. However, with the nonspecific stains the interstitial thickening was very conspicuous. This infiltrate was stained yellow by Van Gieson's solution of trinitrophenol and fuchsin. The staining reactions indicated that at least the denser deposits of the substance belonged to the amyloid group, and that term will be used in the descriptions which follow.

Sections taken from the interventricular septum, the right ventricle and the auricles showed similar infiltration by amyloid. In the septum there was a little interstitial fibrosis.

The other organs studied, including the lungs, liver, kidneys, adrenals, urinary bladder, prostate, seminal vesicles, spleen, pancreas, tracheobronchial lymph nodes, thyroid, diaphragm and femoral nerve, showed marked infiltration and thickening of the media of arterioles and venules by amyloid. However, no significant amounts were deposited about the sinusoids of the liver, around the glomerular capillaries of the kidneys, between the cords of the adrenal cortex and in the corpuscles of the spleen. The diaphragm showed a moderate number of fibers which had an opaque appearance, had lost striations and were relatively acidophil. There was also a little poorly defined amorphous substance between the fibers.

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8. Lillie, R. D., and Pasternack, J. G.: J. Tech. Methods **15**:65, 1936.

8a. Goldner, J.: Am. J. Path. **14**:237, 1938.

Though the walls of some of the larger veins exhibited small qantities of amyloid, the larger arteries showed none except a little deposited in the vasa vasorum.

The following diagnoses were made: generalized vascular amyloidosis; cardiac vascular and interstitial amyloidosis, with myocardial degeneration; chronic passive congestion of the lungs, liver and spleen; infarction of the lungs, kidneys and spleen; thrombosis (or embolism) of the left common iliac artery.

COMMENT

The myocardial failure in this case appears to have been due to the obliterative stenosis of the smaller divisions of the vascular tree by the amyloid deposits in their walls. The circulation to the myocardial fibers was further impaired by the interstitial deposition of hyaline material. Though there were scattered small nodules of amyloid replacing muscle fibers, there was not shown the extensive nodular infiltration of the myocardium reported by several authors.

In commenting on his case, Larsen [4] stated that the amyloid was always deposited about capillaries and venules of the heart and that it was not found in the walls of the coronary arteries. He expressed the belief that the necrosis and loss of muscle fibers had been due to the obliteration of the capillaries and venules. In the present case the arterioles and venules were equally affected. Arteriolar obliteration very probably caused the development of myocardial failure and death without gross nodular amyloidosis of the myocardium.

The widespread deposits in the walls of the smaller vessels in all organs studied is of interest. It is noted that the usual deposition in the liver, spleen, adrenals and kidneys did not take place. Koletsky and Stecher [7] emphasized the observation that in primary amyloidosis, instead of the deposits in the usual organs, the tongue, heart, stomach, intestine and skeletal muscle are most frequently affected.

The hyaline material found enveloping the myocardial capillaries and muscle fibers was easily demonstrated by nonspecific stains, but only rarely did it show any metachromatic staining characteristics when stained by methyl violet or gentian violet. Kerwin [1] mentioned the difficulty experienced in obtaining typical amyloid reactions in the heart in his case and quoted Lubarsch [9] as stating that there may be a failure in usual staining reactions in atypical amyloidosis. He also calls attention to a difficulty reported by Smetana [10] in obtaining stains of recently formed amyloid produced experimentally.

SUMMARY

The case of a 56 year old white man who died of myocardial failure and cardiac asthma is reported. Autopsy showed stenosing amyloidosis

9. Lubarsch, O.: Virchows Arch. f. path. Anat. **271**:867, 1929.
10. Smetana, H.: Bull. Johns Hopkins Hosp. **37**:383, 1925.

of the small coronary vessels and interstitial deposition of a hyaline substance in the myocardium, as well as generalized amyloidosis of the small arteries and veins. There was no associated chronic disease.

Dr. W. W. Nesbit and Dr. H. R. Ostrander, of the United States Marine Hospital, Detroit, supplied the clinical data on the case reported, and Dr. Edgar H. Norris, professor of pathology, Wayne University Medical School, Detroit, gave aid in obtaining the photomicrographs.

WAVE MECHANICS OF SMOOTH MUSCLE ACTION

XV. EXPERIMENTAL MULTIPLE REFLECTIONS BETWEEN INTESTINAL LIGATURES TRANSFORM TRAVELING INTO STATIONARY MICROPRESSURE WAVES IN SMOOTH MUSCLE

EBEN J. CAREY, M.D.
MILWAUKEE

The physical nature of the microscopic structural changes associated with the physiologic and pathologic contractions of smooth muscle is unknown. Excellent observations on fixed material have been recorded by McGill [1] and like observations on living isolated smooth muscle cells by Margaret and Warren Lewis.[2] McGill's description of swollen contraction nodes was confirmed by Lewis and Lewis. They stated, furthermore, that the narrow internodal region in the living cell is under longitudinal tension or stretch. The Lewises were unable to observe the nuclear changes within the active contraction nodes. This was probably due to the low refractive index of the nucleus as compared with that of the active living cytoplasm in the contraction node. The physical significance, however, of the alternate nodes and internodes in contracting smooth muscle is unknown.

The object of this paper, therefore, is to present experimental evidence that points to an *associated micropressure wave mechanics of protoplasmic activity inseparable from the colloidal physical and chemical reactions of smooth muscle contraction.*

These explosive micropressure waves of confined colloidal chemical changes underlie both the gross and the microscopic structural displacement of living muscle protoplasm. The absence and the presence of pressure waves, therefore, determine the variable and reversible or irreversible differential spatial distribution and physical attributes of both the cytoplasm and the nuclei of smooth muscle during rest and motion. In resting smooth muscle these waves are relatively absent.

From the Department of Anatomy, Marquette University School of Medicine.

These investigations were carried out with the aid of a grant for research to the Department of Anatomy of the Marquette University School of Medicine by the Committee on Scientific Research of the American Medical Association. A part of the histologic evidence presented in this paper was demonstrated at the April 1939 meeting of the American Association of Anatomists at Harvard University (Anat. Rec. **73**:11 and 62, 1939).

1. McGill, C.: Am. J. Anat. **9**:493, 1909.
2. Lewis, M. R., and Lewis, W. H.: Am. J. Physiol. **44**:67, 1917.

During contraction the colloidal particles are aggregated in the regions of greatest intensity of the longitudinal waves, namely, the zones of condensation. The absence and the presence of the two regions of (1) nodal compressional condensation and (2) internodal tensional rarefaction of the longitudinal pressure waves determine the reversible alterations in the internal structure from the state of rest to that of motion, respectively, of smooth muscle.

It is the gradient pressure and power of superposed multiple microscopic traveling and overlapping waves of internal compression associated with chemical reactions that push the bolus and partly determine the directional stabilization in the action of smooth muscle. This is in harmony with the metabolic gradient theory of Alvarez [3] regarding the directional stabilization of motion of the peristaltic waves of the intestines. The grossly visible propulsive peristaltic waves of the intestine are resultants of multiple overlapping and microcompressional waves by algebraic addition. By experimentally changing the resultant traveling into a spastic stationary system of intestinal peristaltic waves, by multiple reflections and constructive interference, between ligatures, the components of the micropressure waves become separated, fixed and oriented in space. The structural identification of the parts of the micropressure waves in smooth muscle under the described experimental conditions is therefore clearly evident in histologic preparations. An irreversible, fixed dynamic ileus of the intestinal segments is produced in these experiments.

MATERIALS AND METHODS

Sixty-five etherized guinea pigs, weighing about 250 Gm. each, are used in these experiments. A midline ventral abdominal incision is made. The large and small intestines are exposed, with blood supply intact, and placed between gauze sponges saturated with Ringer's solution warmed to 37 C. Fecal pellets are gently pushed above and below the sites selected for encirclement of the large intestine with tightly tied catgut ligatures placed 1 inch (2.5 cm.) apart. At variable sites between the ligatures 0.5 cc. of Ringer's solution at 37 C. is injected into the intestinal lumen in each of fifty segments, in 10 animals; 0.5 per cent acetylcholine in 0.5 cc. of Ringer's solution is injected into the lumens of fifty segments, in 10 animals; 0.5 per cent pilocarpine in 0.5 cc. of Ringer's solution is injected into the lumens of fifty segments, in 10 animals. A small crystal of barium chloride is placed on the external surface of each of twenty intestinal segments, in 5 animals.

As soon as the opacity indicating intestinal spastic contraction occurs between the ligatures, the segment is excised with the terminal ligatures and placed in Ringer's solution at 5 C. for ten minutes to stop the active state of the muscle; it is oriented and tied on 1 by 3 inch (2.5 by 7.5 cm.) microscopic glass slides, then fixed in a 10 per cent solution of neutral formaldehyde U. S. P. in 20 per cent dilution, for twenty-four hours. Pieces one-half (1.2 cm.) inch long are

3. Alvarez, W. C.: The Mechanics of the Digestive Tract, New York, Paul B. Hoeber, Inc., 1929, p. 55.

dehydrated in alcohol, cleared in xylene, embedded in paraffin and cut serially in both cross and longitudinal sections at 4 to 6 microns. The sections are variously stained, but hematoxylin and erythrosin give good uniform results.

For inactive controls 0.5 per cent atropine in 0.1 cc. of Ringer's solution is injected into the lumens of fifty segments, in 5 animals. In addition controls are obtained by killing guinea pigs with ether. Then the animals are placed in a refrigerator at 5 C. for twenty-four hours before the abdomen is opened and the intestinal segments excised for microscopic preparation. Irritability is therefore destroyed before the intestines are handled and placed in a fixative. Control traveling waves are obtained and fixed by injecting subcutaneously 5 cc. of 1 per cent pilocarpine in Ringer's solution at 37 C. in 5 etherized animals. The abdomen is then opened. The entire animal with stimulated intact intestines is submerged and fixed in solution of formaldehyde U. S. P. diluted 1:10, for twenty-four hours.

The external surface of the exposed intestinal segments between ligatures, with blood supply intact, is observed under Ringer's solution at 37 C. in 20 animals, with the Ultrapac microscope and water immersion lenses. The various experimental procedures enumerated are repeated. Direct observations of the peristaltic waves through the serosa are made. The direct conversion of traveling peristaltic waves into a stationary system is observed between the intestinal ligatures in both the large and the small intestine.

The gizzard of the pigeon is studied in microscopic section in order to determine the histologic changes following inactivity and activity. The inactive gizzard muscle is obtained by killing the bird with magnesium sulfate, 2 Gm. per kilogram of body weight. The birds die in one to three hours after receiving the injection. They are then placed in the refrigerator at 5 C. for twenty-four hours prior to fixation. The active gizzard muscle is obtained by immersing the gizzard in Ringer's solution at 39 C. for ten minutes prior to fixation.

EXPERIMENTAL RESULTS

The direct conversion of traveling propulsive peristaltic waves into short nonproductive stationary waves of spastic contraction is observed with the aid of the Ultrapac microscope on the external surface of the intestinal segments between the ligatures. Shortly after the injection of 0.5 cc. of 0.5 per cent acetylcholine in Ringer's solution into the lumen of the segment short traveling incident waves radiate both ways from the site of the injection. These fine waves produce localized rhythmic contractions. Multiple reflections of the fine ripples occur back and forth between the ligatures.

This is comparable to observations made with the unaided eye on the external surface of the intestine by Engelmann,[4] Cannon,[5] Meek,[6] Hyman,[7] Child,[8] Alvarez,[3] and others. Alvarez used the word "metabolism" and the term "metabolic or physiologic gradient" in reference to

4. Engelmann, T. W.: Arch. f. d. ges. Physiol. **2**:259, 1869.
5. Cannon, W. B.: Arch. Int. Med. **8**:419, 1911.
6. Meek, W. J.: Am. J. Physiol. **24**:232, 1911.
7. Hyman, L. H.: Biol. Bull. **37**:388, 1919.
8. Child, C. M.: Biol. Bull. **39**:147, 1920.

the directional stabilization of the peristaltic waves in the same sense as Hyman [7] and Child,[8] respectively, used them. They are indicators, respectively, of the sum of all the energy-producing and substance-producing processes and the differential rates of chemical change between two localities in space. Oxidative processes are eventually and ultimately associated with all of the other physical and chemical factors in the living protoplasmic system even though for short periods certain chemical reactions are anaerobic in muscle.

There is sudden cessation of intestinal movement when 0.5 cc. of 0.5 per cent acetylcholine in Ringer's solution is injected into the lumen of a segment of the large intestine 1 inch long. Superpositional constructive interferences of incident and reflected waves produce a strong tonic contraction. When spasticity occurs, the intestinal segment immediately becomes opaque and relatively bloodless. The two intestinal ligatures act like mirror obstacles and cause multiple reflections in a confined localized space of the intestinal segment. The shape of the intestinal segment after fluid has been injected into the lumen may be uniformly cylindric, dumbbell, fusiform, or Indian club shaped. This shape is dependent on the site and on the rapidity of the injection of fluid into the lumen between the two ligatures. Long overlapping constrictions of the traveling wave system are 1 to 8 mm. in length. They have a relatively smooth external surface. When they are converted into the stationary system of waves, there is a subdivision into microscopic waves, which appear as convexities on the external surface of the intestine. These fine convexities in the spastic intestinal segments remain relatively fixed, and the distance from the crest of one convexity to the crest of the next is 10 to 120 microns.

HISTOLOGIC RESULTS

The inner, closely wound spiral muscle coat (Carey;[9] Reid, Ivy and Quigley [10]) is not as favorable a location in which to observe the histologic changes of smooth muscle contraction as the outer, open, elongated spiral layer. The reason for this is the difficulty of cutting the fibers of the inner layer parallel to their long axis. The muscle fibers of the outer layer are, therefore, relatively more longitudinal and parallel to the long axis of the intestine. The muscle fibers of the outer layer are, therefore, relatively more longitudinal and parallel to the long axis of the intestine. Cleancut patterns of alternate condensations and rarefactions of the cytoplasm and nuclei are best observed in longitudinal sections of the intestine (figs. 27, 28 and 29) as nearly parallel as possible to the longitudinal direction of the fibers in the outer muscle

9. Garey, E. J.: Anat. Rec. **21**:189, 1921.

10. Reid, P. E.; Ivy, A. C., and Quigley, J. P.: Am. J. Physiol. **109**:483, 1934.

layer. Since the cutting of the sections in a favorable plane is a trial and error proposition, many thousands of sections must be made and studied in order to obtain favorable patterns for record.

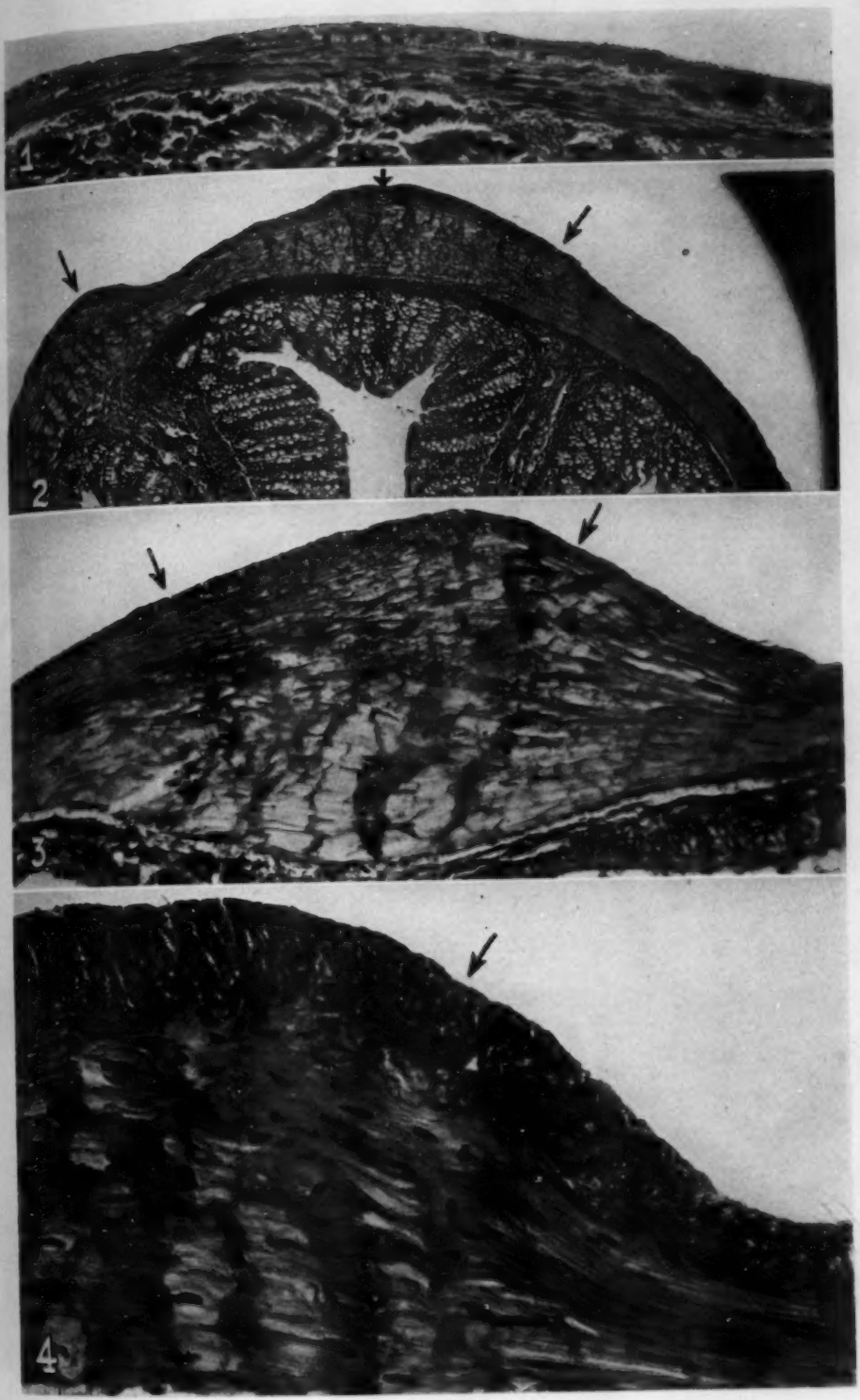
Relatively resting smooth muscle is observed in cross (figs. 1, 8 and 24) and longitudinal (fig. 26) sections of the large intestine. In the active muscle of the inner coat there are localized lateral expansions designated by arrows in figures 2, 3 and 4. Underlying these expanded areas are irregular alternate dark and light stripes of the cytoplasm. The orientation of these stripes is better observed in the outer muscle layer (figs. 27, 28 and 29). In the dark stripe many nuclei are round and uniformly deeply stained, whereas in the light one they are elongated and granular. This becomes clear when the outer coat of muscle is observed in longitudinal sections of the intestine (figs. 27, 28 and 29).

Attention is especially directed to this histologic evidence in figure 29. The nuclei in the dark stripe of the cytoplasm (figs. 5, 13, 14, 20, 21, 22 and 23) are rounded, oval, dumbbell and arrow-head in shape and deeply stained. In the light stripe (fig. 29) they are elongated and segmented by granules. A single nucleus may have many deformations, dependent on the way it straddles either a dark and a light stripe or parts of each stripe of the cytoplasm. These differential deformations of the nuclei are related to the spatial distribution of the active cytoplasm of smooth muscle into dark and light stripes of condensation compression and rarefaction tension, respectively. The nuclei appear to be passive and are deformed by the active cytoplasmic pressure waves. This agrees with the observations of Lewis and Lewis,[2] who stated (p. 72): "Within the nucleus, nucleolus or the mitochondria there was no change which could be considered a causal factor of the contraction or relaxation."

The spatial distribution of the cytoplasmic colloid in contracting smooth muscle under the influence of standing pressure waves is depicted in figures 9, 10, 11 and 12. The transformation of a resting medium (fig. 15) into a traveling (fig. 16) and into a standing wave system (fig. 17) is clearly seen in the rubber tube experiment. The state of resting muscle of the inner muscle coat (fig. 18) is contrasted with the histologic changes produced by an overlapping traveling (fig. 19) and a standing system of waves (fig. 20). In the latter system the component parts, namely (1) condensation and (2) rarefaction of the frozen longitudinal pressure waves, are clearly evident whereas in the former the histologic picture is blurred by the overlapping of the elements of the wave system. The contrasting pictures of the structural changes during smooth muscle contraction in the longitudinal (fig. 6) and the cross section (fig. 7) are clearly evident. The zones of condensation and rarefaction are striking in the cross section (fig. 7) of the inner smooth muscle layer.

EXPLANATION OF FIGURES 1, 2, 3 AND 4

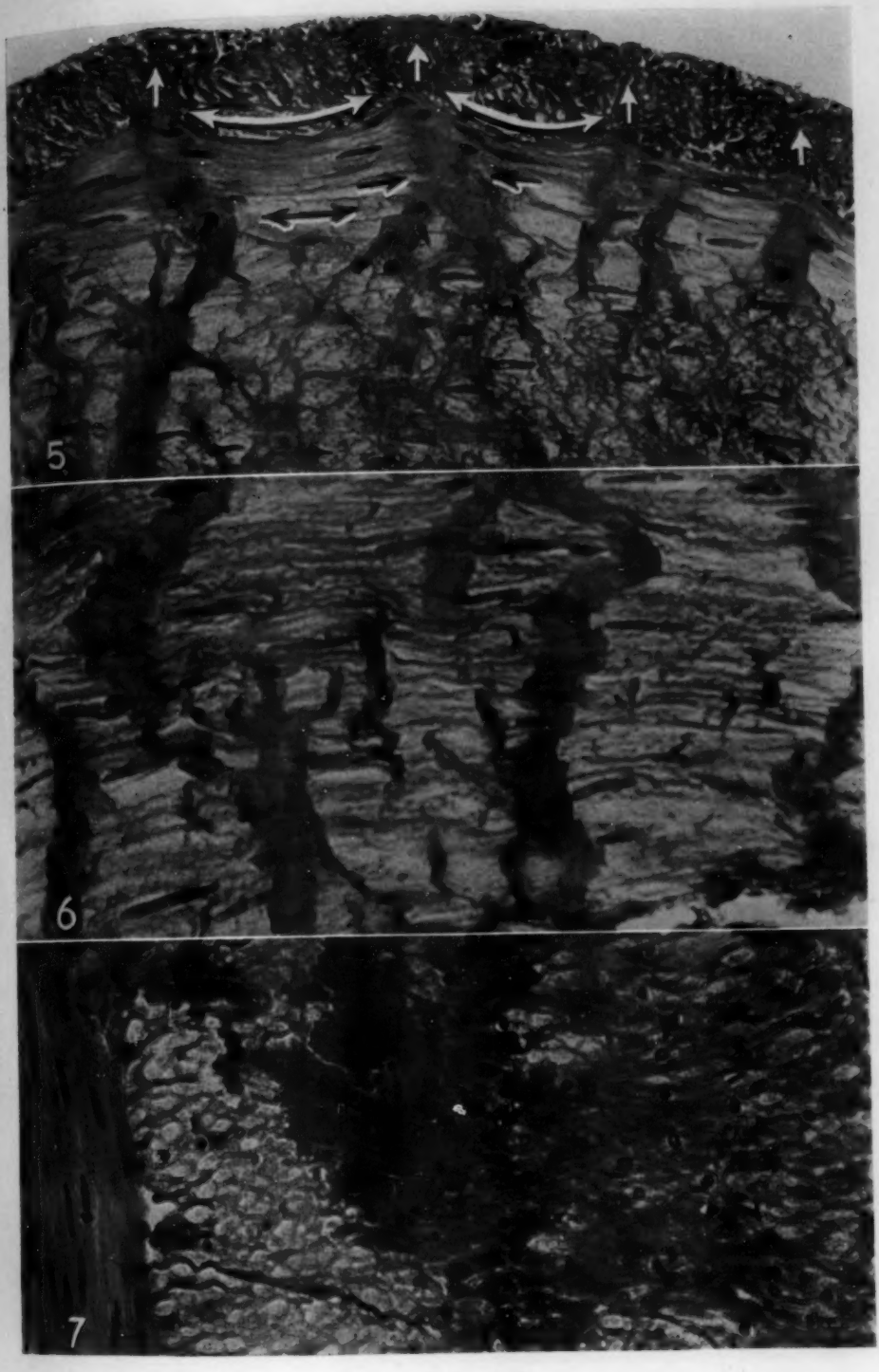
Parts of the cross sections of the muscle wall of the large intestine of the guinea pig. Figure 1 shows resting inner smooth muscle layer; × 154. The active inner smooth muscle layer (figs. 2, 3 and 4, × 38.5, 23 and 423, respectively), with regions of lateral expansion marked with arrows, has dark and light stripes of the cytoplasm. In the dark stripe the nuclei and cytoplasm are condensed by pressure. In the light stripe the nuclei and cytoplasm are stretched by longitudinal tension. The orientation of the dark and light stripes is better observed in the longitudinal sections of the outer muscle layer (figs. 27, 28 and 29) than in the transverse sections of the intestine, which cut the inner, closely wound spiral muscle layer at a tangent in various degrees.



Figures 1, 2, 3 and 4

EXPLANATION OF FIGURES 5, 6 AND 7

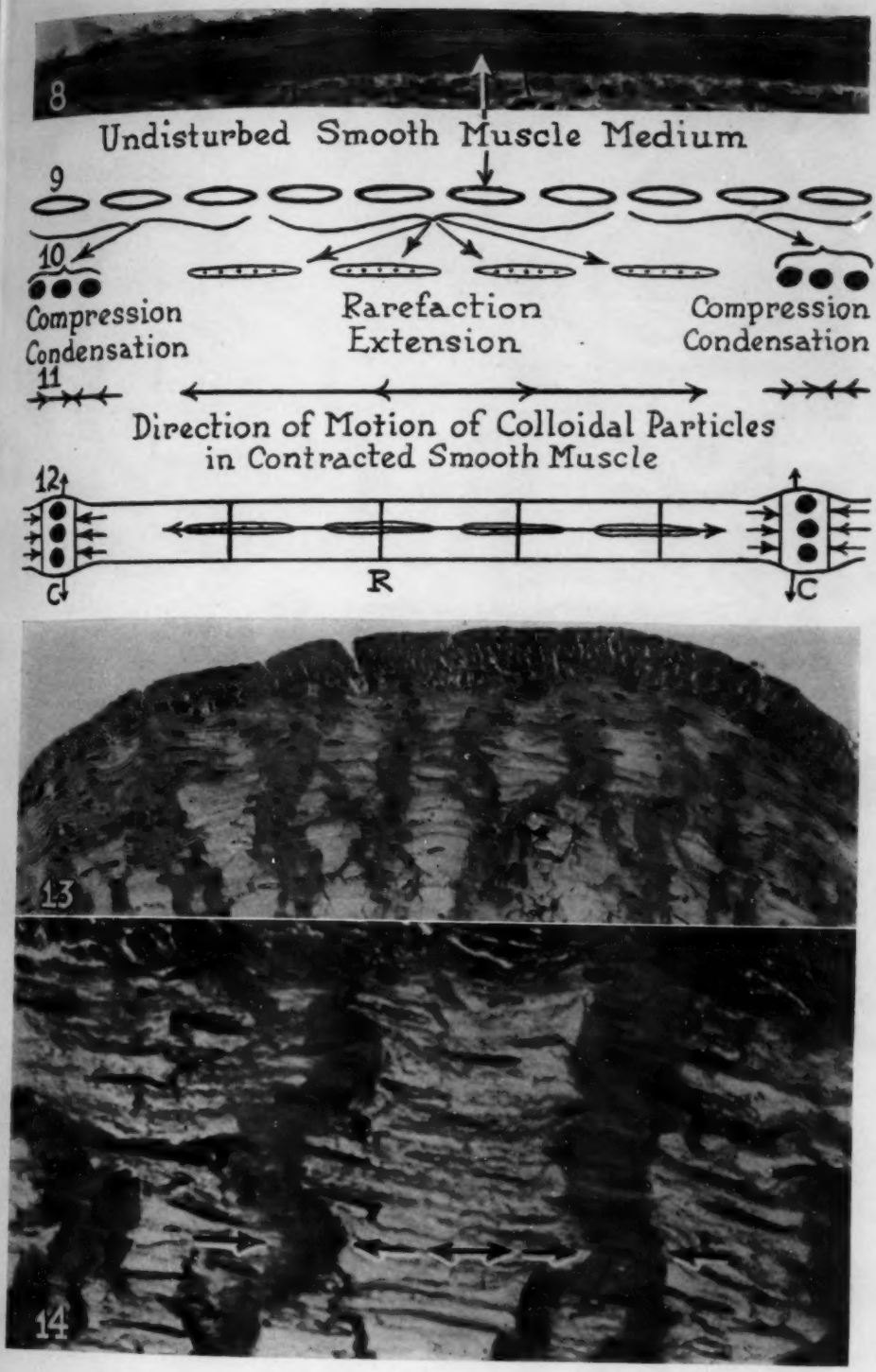
Parts of cross and longitudinal sections of the muscle wall of the large intestine of the guinea pig. Figure 5 shows the lateral expansions of the dark stripe marked by arrows. These dark stripe zones are also designated as contraction nodes with dark condensed nuclei. The lightly stained and rarefied internodal regions under stretch have elongated granular nuclei and fibrillated cytoplasm. The various deformations of the nuclei are clearly evident in both figure 5 (× 423) and figure 6 (× 423). Figure 7 shows a longitudinal section of the large intestine of the guinea pig; × 423. The outer layer to the left is relatively at rest and cut in longitudinal section. The larger region to the right is the inner muscle layer cut in transverse section. The dark cytoplasm and nuclei are in transverse section through the condensed contraction node. The lighter regions are in transverse sections through the rarefied internodes of the inner muscle layer of the large intestine.



Figures 5, 6 and 7

EXPLANATION OF FIGURES 8, 9, 10, 11, 12, 13 AND 14

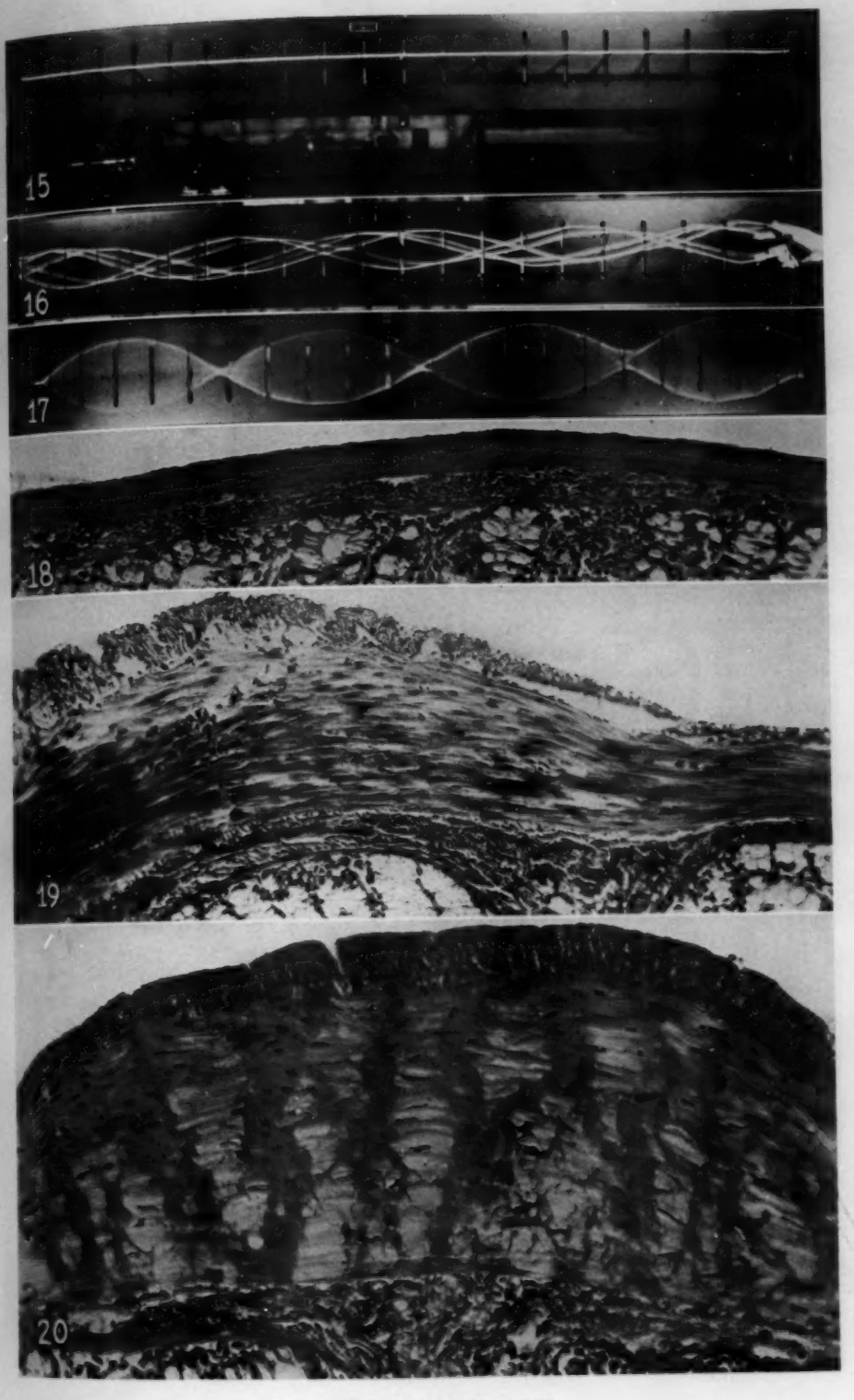
Parts of cross sections of the muscle wall of the large intestine of the guinea pig. Figure 8 shows resting inner smooth muscle layer; × 231. Figures 9, 10, 11 and 12 are diagrams of the changes in shape of the particles of the muscle colloid from the resting condition to that of active contraction under the influence of longitudinal pressure waves inseparably associated with the colloidal chemical changes during muscle contraction. The zones of condensation and rarefaction are the parts of each longitudinal wave of compression within the muscle. Figures 13 and 14 (× 231; × 538.5) represent transverse sections through parts of the muscle coat of the large intestine showing the spatial distribution and deformation of the cytoplasm and nuclei into longitudinal pressure waves with a dark stripe of condensation and a light one of rarefaction.



Figures 8, 9, 10, 11, 12, 13 and 14

Explanation of Figures 15, 16, 17, 18, 19 and 20

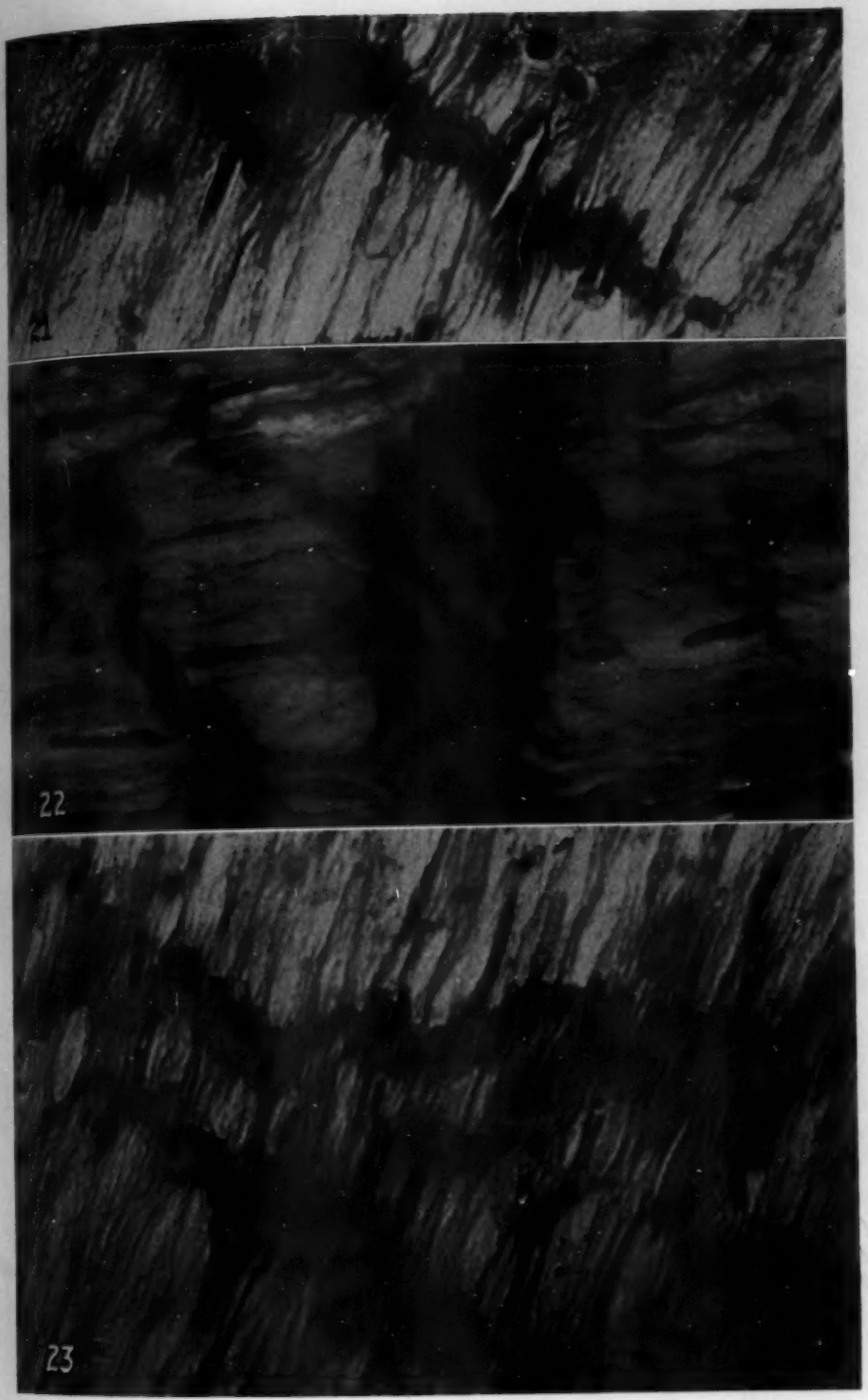
Figure 15 shows a wave apparatus. This demonstration model of transverse wave motion consists of a flexible rubber tube, 6 feet (183 cm.) long, stretched horizontally and supported by twenty cross arms (Carey [12]). Each cross arm is connected to a ball bearing mounted on a long steel shaft around which the cross arms are free to oscillate in vertical planes. The rubber tube is loaded with shot and balanced by counterweights attached to the back end of each cross arm. The rubber tube as a transmission medium of vibration is at rest. Figure 16 shows the rubber tube experimentally activated by the motor on the left. The end of the tube at the right is free to oscillate. This freedom of motion produces a traveling system of waves. The traveling waves produce an overlapping of parts, with lack of cleancut definition of nodes and internodes. Figure 17 shows the rubber tube experimentally activated by the motor which is on the right. The end of the rubber tube on the left is fixed. Multiple reflections from the fixed end produce a standing system of waves by superposition and constructive interference with definitely cleancut nodes and internodes. The micropressure waves in the intestine are dominantly of the longitudinal type. Figures 18, 19 and 20 show parts of cross sections of the muscle wall of the large intestine of the guinea pig. Figure 18 represents the resting inner smooth muscle layer (× 231); figure 19, a fixed traveling system of contraction waves originally free to travel but now "frozen," with overlapping of nodes and internodes in the inner smooth muscle layer of the large intestine (× 231); figure 20, stationary pressure waves transformed between intestinal ligatures from a traveling system of peristaltic waves; (× 231). A "frozen" system of waves consisting of condensed nodes and rarefied internodes are more definitely separated, oriented and fixed in space than those of the traveling system illustrated in figure 19. This is apparently an interference phenomenon which may be experimentally produced in muscle and which is comparable to that in the rubber tube, in the conversion of a traveling into a standing system of waves.



Figures 15, 16, 17, 18, 19 and 20

Explanation of Figures 21, 22 and 23

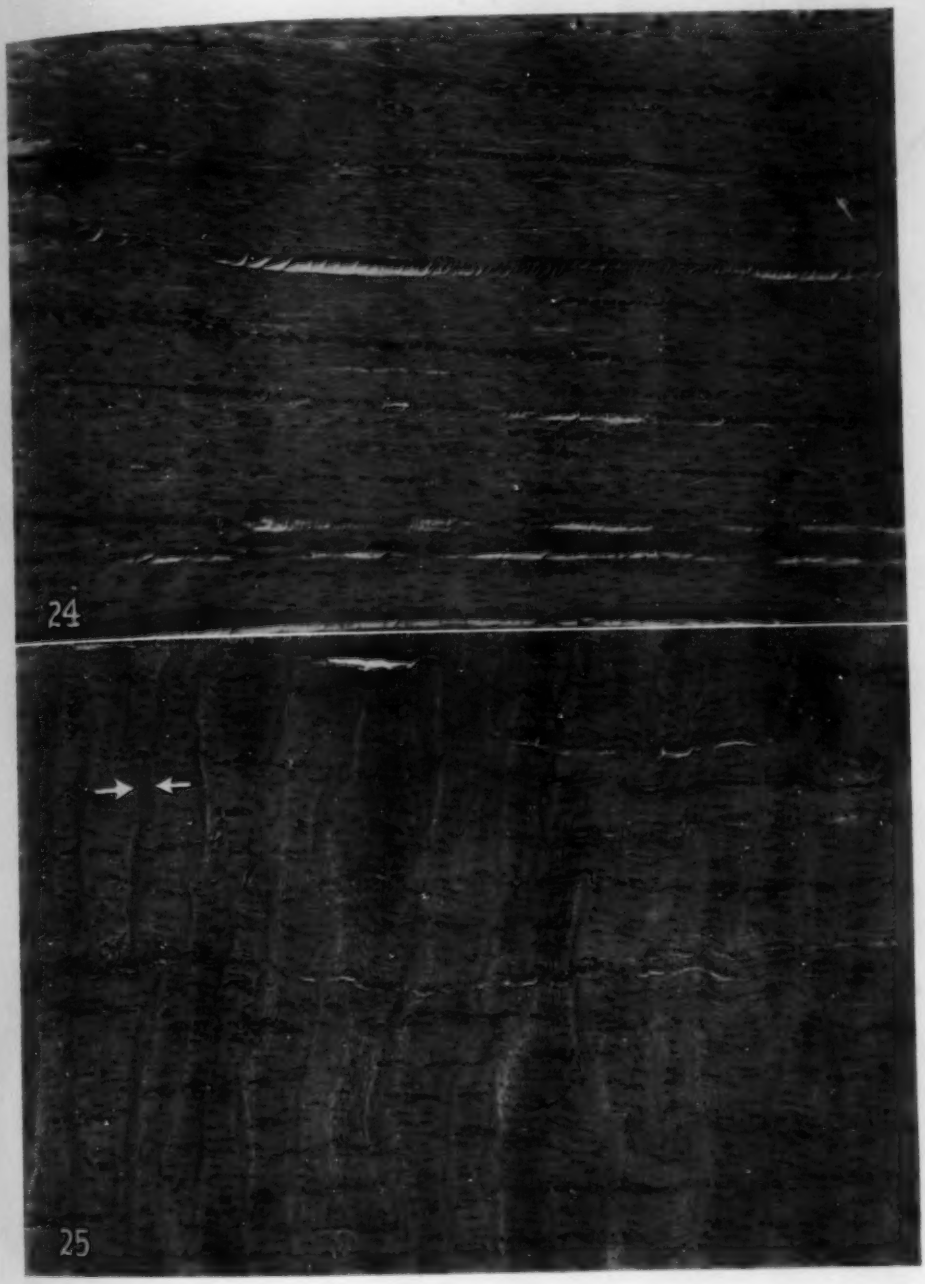
Parts of cross sections of the muscle wall of the large intestine of the guinea pig. Figures 21, 22 and 23 (all × 769) illustrate clearly the variable deformations of the nuclei in the zones of condensation and rarefaction. The nuclei are round, oval, tapering or compressed, and densely stained in the zone of condensation, which is the dark stripe. The zone of rarefaction is the lightly stained region. In the rarefied zone the nuclei are greatly elongated, stretched and granular. In figure 21 there are three nuclei at the right end of the dark stripe of condensation which are of dumbbell shape. In the centers of these nuclei there is a biconcave condensation, very deeply stained. The ends of these same nuclei are globular, lightly stained and granular. The various changes in shape of the nuclei are clearly evident in these various figures. The cytoplasm appears to be the region of dominant activity in smooth muscle contraction. The nuclei are relatively passively deformed by the two components of the contraction cytoplasmic wave, namely (1) the condensed contraction node and (2) the tension internode.



Figures 21, 22 and 23

EXPLANATION OF FIGURES 24 AND 25

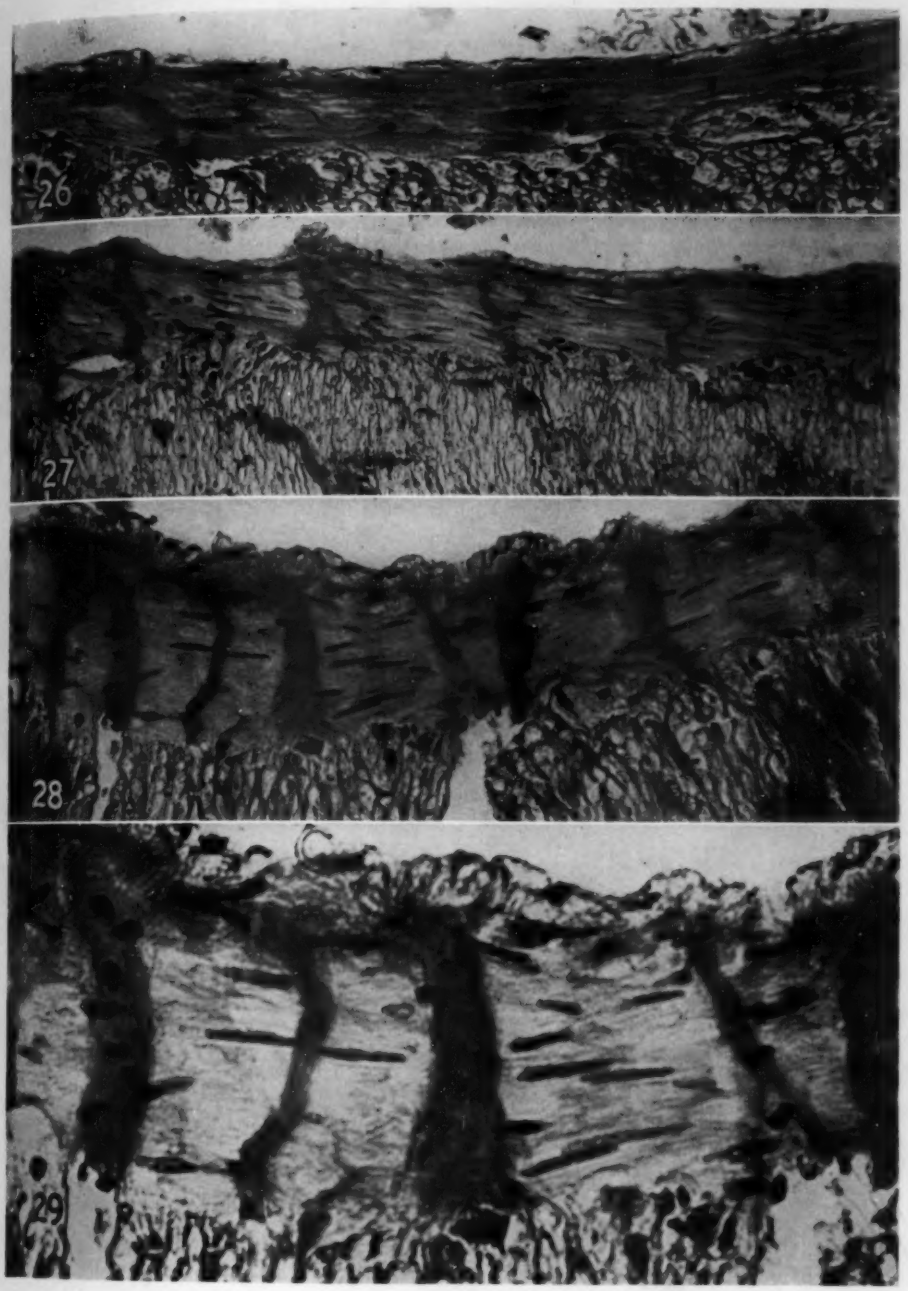
Figure 24 shows inactive muscle of the gizzard of the pigeon; (×269). Experimentally it has been placed in a state of extreme inactivity by dehydration through an injection of magnesium sulfate, 2 Gm. per kilogram of the body weight of the pigeon. There are no contraction waves with zones of condensation and rarefaction. Figure 25 shows a section of the gizzard of the pigeon which was placed in Ringer's solution at 39 C. for ten minutes prior to fixation; (×269). Zones of nodal condensation and internodal rarefaction of contraction waves are clearly evident. These microscopic waves are histologic indicators of the dynamic state of the muscle protoplasm. There appears to be a correlation between the number of these waves and the rate of the chemical reactions associated with muscle contraction and under the regulation of temperature.



Figures 24 and 25

EXPLANATION OF FIGURES 26, 27, 28 AND 29

Parts of longitudinal sections of the muscle wall of the large intestine of the guinea pig. Figure 26 shows resting outer smooth muscle layer (× 269). Figure 27, stationary pressure waves experimentally produced in the outer smooth muscle layer (× 269). The convexities of the outer wall overlying the contraction nodes of pressure condensation and the concavities related to the tension internodes under stretch are clearly evident. Figures 28 and 29 (× 461.5; × 846) illustrate clearly the characteristic deformations of the nuclei and cytoplasm in the zones of condensation and rarefaction of the stationary longitudinal pressure waves associated with the physicochemical changes of spastic muscle contraction. The nuclei are dark, rounded, pyknotic and condensed in the dark stripe zones of condensation. The cytoplasm is likewise clearly condensed and deeply stained in the zones of condensation, called at the present time the contraction nodes. The lightly stained tension internode has rarefied cytoplasm which is definitely fibrillated. This internodal region is under a longitudinal tension or stretch. The nuclei are elongated and granular in this internodal zone of stretch. The nuclei appear to be passively deformed by the two parts of the waves of internal compression inseparably associated with the colloidal physicochemical changes of smooth muscle contraction.



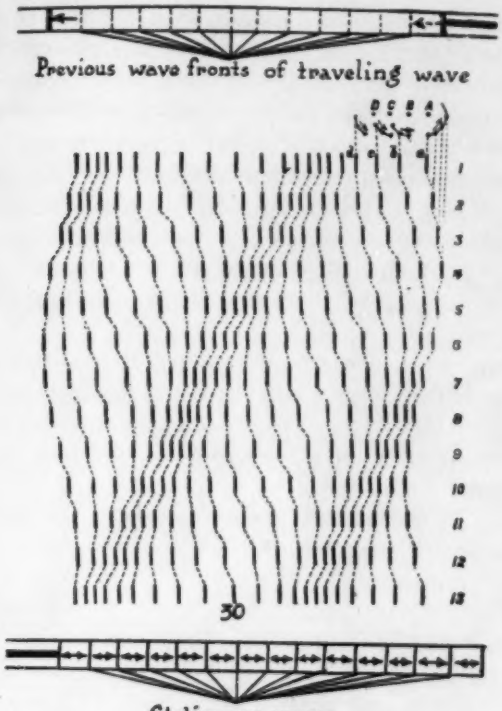
Figures 26, 27, 28 and 29

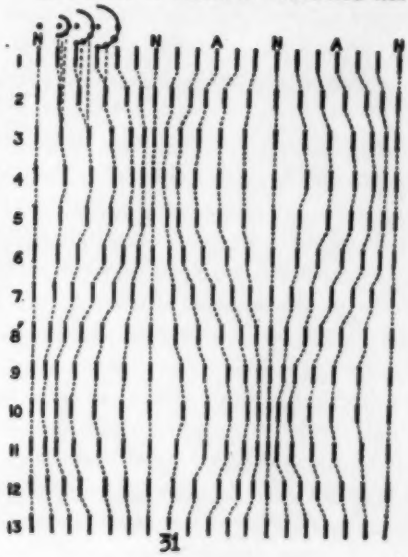
Explanation of Figures 30 and 31

Figure 30 shows progressive movements and distribution of the particles of a medium traversed by a single wave traveling from right to left. The vertical lines in each row represent plane surfaces seen edgewise, which were equidistant in the undisturbed medium. The same medium always remains between the same two planes. The successive rows 1 to 13 represent successive stages of a movement in which each of the vertical lines vibrates harmonically a specific distance to right and left of a fixed point. Each line of a row from above downward and from right to left is a little behind the line to the right of it in its movement. The mean positions of the lines are equidistant, but the lines themselves are at any one instance of time closer in some places and farther apart in others. These conditions are found farther to the left at each successive stage of the motion. If any one line is traced, by the dotted lines, from one row to another, it will be seen that when movement is to the left, i. e., in the same direction as the traveling waves of condensation and rarefaction, the line is nearest to its neighbors. When the line is moving fastest to the right it is in the most rarefied region.

Figure 31 is a diagram of movements in successive periods of time in the same medium traversed by stationary undulations. The vertical lines in rows 1 to 13 represent plane surfaces seen edgewise. These are equidistant in the undisturbed medium. The same medium is always between the same two vertical lines. The lines vibrate harmonically about their mean positions, those at the antinodes (A) have the greatest amplitude of vibration, and minimum pressure and temperature; those at the nodes (N) have zero amplitude, and maximum pressure and temperature. They all cross their mean positions together, but those on opposite sides of the same node are moving in opposite directions. In successive lines it is apparent that the medium shuttles backward and forward between relatively fixed planes, the nodes.

The fundamental spatial and temporal differences in the distribution of the particles of the medium when the latter is traversed by either a progressive traveling or a stationary system of waves are herein schematized. In the traveling wave all parts of the medium move the same distance but at different times. In the stationary system of waves all parts of the medium move at the same time but different distances. Differences in phase and amplitude of stationary waves are reflected in the distribution of the particles of the medium through different distances. (Diagrams modified after Catchpool: Textbook of Sound, London, Clive, 1917.)





Figures 30 and 31

The dark stripe region of cytoplasmic condensation has the following microscopic characteristics: (1) anisotropy; (2) presence of inorganic ash (Carey and Zeit [11]); (3) compression of cytoplasm with close packing of colloidal particles; (4) rounding, deformation and compression of densely stained nuclei; (5) lateral expansion of cytoplasm. The light stripe region of rarefaction is characterized by: (1) isotropy; (2) relative lack of ash (Carey and Zeit [11]); (3) rarefaction of loosely packed and lightly stained muscle colloids, with fibrillation by stretch or tension; (4) stretching of granular nuclei to almost four times the length of those in the dark condensed stripe; (5) longitudinal tension of cytoplasm.

In the large intestine of the guinea pig the isolated group of the contraction waves that form a lateral expansion of the intestine seldom extend entirely around the circumference of the intestine (fig. 2). In a traveling peristaltic wave the cytoplasm of the cell may be either totally or partially under contraction.

The average of five hundred measurements of the nuclei in inactive smooth muscle is: 51 microns long and 9 microns wide. The average of five hundred measurements of the nuclei in the zone of condensation of spastic contraction of smooth muscle is: 18 microns long and 15 microns wide. The average of five hundred measurements of the nuclei in the zone of rarefaction of spastic contraction of smooth muscle is: 65 microns long and 5 microns wide. There are therefore an increase in the length and a decrease in the width of the nuclei in the zone of rarefaction or stretch, and a decrease in length and an increase in width of the nuclei in the zone of condensation or pressure of smooth muscle in spastic contraction, over the measurements of the nuclei of the inactive muscle.

The inactive smooth muscle of the pigeon's gizzard (fig. 24) is contrasted with the active one (fig. 25). The micropressure waves in the active gizzard muscle are an index of the raised level of the dynamic state, or of an acceleration in the chemical changes of metabolism, under the influence of an elevation in the temperature. The acceleration in the rate of chemical reactions caused by the elevation in the temperature is accompanied by an increase in the number of micropressure waves, histologically evidenced by the close periodic spatial distribution of the cytoplasm into alternate condensed (dark) and rarefied (light) stripes and corresponding deformations of the nuclei.

The fundamental spatial and temporal differences in the distribution of the particles of the medium is schematized in figure 30, traversed by a traveling wave, and in figure 31, traversed by a standing or stationary system of waves. In the traveling wave all parts of the medium

11. Carey, E. J., and Zeit, W.: Proc. Soc. Exper. Biol. & Med. **41**:31, 1939.

move the same distance at different times. In the stationary system of waves all parts of the medium move at the same time but different distances. Differences in phase and amplitude of stationary waves are reflected in the distribution of particles of medium through different distances.

COMMENT AND SUMMARY

Experimental microscopic evidence is presented of changes in the differential spatial distribution, deformation and physical, mechanical and staining qualities of the cytoplasm and nuclei during smooth muscle contraction. Stationary intestinal waves, experimentally transformed from traveling waves, are similar to those of inanimate matter in association with phase differences of stationary transverse or longitudinal pressure waves, such as sound or supersonic waves (Carey [12]). The number of micropressure waves associated with the colloidal chemical changes of active protoplasm confined in a relatively constant volume appears to be an index of the dynamic state, or metabolic activity, of smooth muscle during contraction.

The variable reversible rates of the chemical reactions in smooth muscle during rest and motion appear to be associated with variable wavelengths of the micropressure waves. The increased momentum of smooth muscle motion has a complementary group of waves of micropressure. The wavelength inversely corresponds to muscle momentum. When there is a greater degree of muscle momentum and frequency of colloidal chemical change, there is a corresponding shortening of the wavelength, with a finer cytoplasmic grating of dark and light stripes.

In experimental stationary waves produced between two ligatures around the intestine alternate dark and light stripes occur in the smooth muscle cytoplasm. The dark stripe is the compression contraction node, the light one the tension internode of one complete contraction wave. This "frozen" structural expression of this immaterial contraction wave of change is demonstrated microscopically in this paper, for the first time, to have the physical attributes of a longitudinal wave of compression. The colloidal chemical reactions of both aerobic and anaerobic metabolism are associated with a micropressure wave-mechanics of protoplasmic activity of smooth muscle contraction. These explosive micropressure waves underlie the physical changes of protoplasmic activity. Physical confinement and degree of constructive interference determine the microscopic definition and orientation of these microwaves. The frequency of the oscillating colloidal particles that radiate the waves is associated with the rates of the physical and chemical changes of the active cytoplasm. The size of the colloidal

12. Carey, E. J.: Am. J. Anat. **59**:175, 1936.

particles that determines the frequency varies with the chemical change. The site of active contraction is the cytoplasmic micropressure waves. The nuclei are relatively passive and undergo deformation by the components of the microscopic waves of longitudinal pressure.

According to the great French physicist Louis de Broglie,[13] it seems to be definitely established that both matter and light have two aspects. Each may be regarded either as a wave or as a substantive corpuscle. Both matter and radiation, therefore, have a dual nature.

The conclusion is made in this paper that the cytoplasmic living matter of smooth muscle is not only composed of colloidal particles but that these in action are distributed in a manner that structurally expresses underlying associated micropressure waves. The living matter of the cytoplasm of smooth muscle, therefore, seems to have two aspects, namely (1) colloidal particle and (2) the associated micropressure wave.

Mr. Leo Massopust assisted with the photomicrographs.

13. de Broglie, L.: Matter and Light, New York, W. W. Norton & Company, Inc., 1939, p. 48.

CAPILLARY RUPTURE WITH INTIMAL HEMORRHAGE IN THE CAUSATION OF CEREBRAL VASCULAR LESIONS

J. C. PATERSON, M.D.
OTTAWA, CANADA

Capillary rupture with intimal hemorrhage in relation to the precipitation of coronary thrombi has been described in detail elsewhere;[1] the observations reported there, and in part the conclusions, have been confirmed and elaborated on by Wartman,[2] by Winternitz and his co-workers[3] and by others. It has been shown that intimal hemorrhages result from the rupture of capillaries derived from the arterial lumens, not from the backflow of blood through intimal defects as was previously thought. Because intimal hemorrhages are a common finding at the sites of precipitation of coronary thrombi, it appears fairly certain that the two lesions are cause and effect. Recently I have suggested that pulmonary thrombi may sometimes be precipitated by similar intimal hemorrhages.[4]

The purpose of the present paper is to describe intimal hemorrhages due to capillary ruptures in sclerotic cerebral arteries and to discuss the relation of these hemorrhages to certain cerebral vascular lesions, namely, arteriospasm, thrombosis and cerebral hemorrhage.

MATERIAL AND METHOD

Most of the material for this study was obtained at autopsies on 6 patients who had shown clinical signs of cerebral thrombosis. When the thrombus was identified on gross examination, the affected segment of the artery was embedded in one or more blocks and sectioned serially at intervals of about 100 microns. When the thrombus was not evident on gross examination, the entire involved artery was cut into short segments, and these were embedded in bundles and sectioned serially at intervals of about 100 microns. When lesions of interest were noted on microscopic examination, the intervening sections were mounted and stained. In case 1 all of the cerebral arteries and their main branches were studied by serial section, the "bundle" method being used. Most of the sections were stained with hematoxylin and eosin, but occasionally Perle's stain and Masson's trichrome light green stain were used.

From the Department of Pathology of the Ottawa Civic Hospital.

1. Paterson, J. C.: Arch. Path. **22**:313, 1936; **25**:474, 1938; J. A. M. A. **112**:895, 1939.
2. Wartman, W. B.: Am. Heart J. **15**:459, 1938.
3. Winternitz, M. C.; Thomas, R. M., and LeCompte, P. M.: The Biology of Arteriosclerosis, Springfield, Ill., Charles C. Thomas, Publisher, 1938.
4. Paterson, J. C.: Am. Heart J. **18**:451, 1939.

The remainder of the material was obtained from the department of neuropathology of the University of Toronto with the aid of Prof. Eric Linell. It consisted of 3 hemorrhagic lesions of the cerebral arteries, each from a different case. Two were from the basilar artery, and the third was from the left middle cerebral artery. The latter was studied by partial serial section.

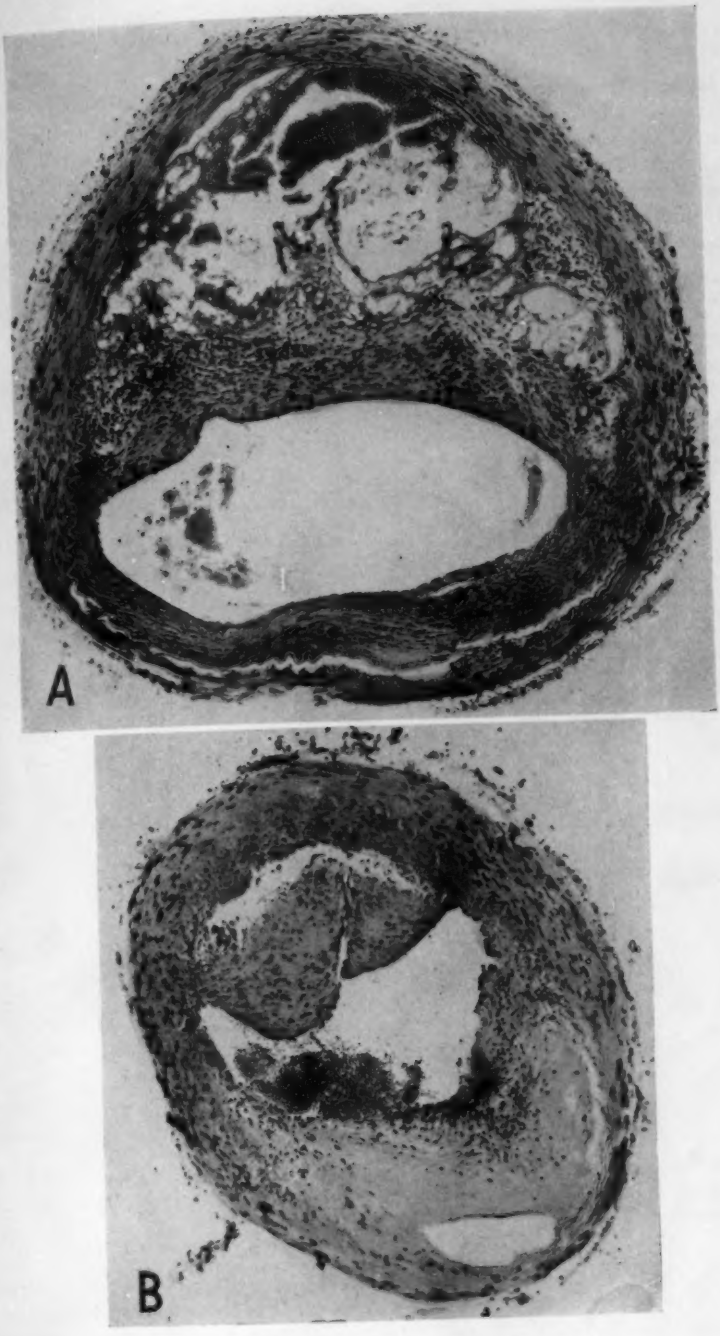
OBSERVATIONS

Intimal hemorrhages of sclerotic cerebral arteries have been found both with and without thrombosis of the adjacent arterial lumens. The hemorrhages were similar in all respects to those described in sclerotic coronary arteries. Serial section through several of them showed the hemorrhage to be confined to the deeper layers of the thickened intima, the more superficial intimal layers and the endothelium being intact (fig. 1 *A*). The intimal hemorrhages were observed only in areas of atheromatous degeneration. In many instances small capillaries lay in proximity to the extravasated blood and to the lumen of the artery. Serial section showed these capillaries to arise from the arterial lumen (fig. 1 *B*). Frequently, in individual cases, intimal hemorrhages were multiple in the cerebral arteries, and sometimes similar hemorrhages were found as well in other arteries of the musculoelastic type. For example, in 1 case there was an intimal hemorrhage at the site of thrombosis in a middle cerebral artery, 11 other intimal hemorrhages in various parts of the cerebral circulation, a large intimal hemorrhage resembling a localized dissecting aneurysm in the abdominal aorta and an intimal hemorrhage of the right coronary artery which produced stenosis of the lumen.

Certain possible sequelae of intimal hemorrhages in sclerotic cerebral arteries were suggested in the series. In the first place, 1 case presented an interesting association of symptoms resembling cerebral arteriospastic attacks, and at autopsy multiple intimal hemorrhages of varying ages were observed in the cerebral arteries. A summary of the history of this case follows:

Case 1.—A 63 year old, obese woman, known to have had diabetes mellitus and hypertension for three years, complained of frequent headaches, fatigue, defective memory and numerous falls over the same period of time. Most of the falls occurred when she tripped over some object, but she had also fallen for no apparent reason when walking on level ground. She had fallen downstairs three times. She was admitted to the hospital with signs and symptoms of cerebral arterial thrombosis. The blood sugar on admission was 314 mg. per hundred cubic centimeters, and the blood pressure was 178 systolic and 118 diastolic. Forty-five days later she died with signs of bronchopneumonia.

The principal abnormalities noted at autopsy were atrophy of the pancreas with fibrosis of the islets of Langerhans, marked atherosclerosis of the aorta, coronary and cerebral arteries, multiple intimal hemorrhages of the aorta, coronary and cerebral arteries, thrombosis of a middle cerebral artery, softening of the right parietal lobe of the brain, thrombosis of the femoral veins and pulmonary embolism.



EXPLANATION OF FIGURE 1

A, photomicrograph of one of the intimal hemorrhages in case 1. The hemorrhage lies close to the media in the outer zone of an atheromatous focus. Hematoxylin and eosin; × 45.

B, photomicrograph of one of the main cerebral arteries in case 1 showing a capillary arising from the arterial lumen and traversing the thickened intima. Hematoxylin and eosin; × 45.

Each of the coronary arteries showed numerous points of stenosis due to the presence of atheromatous plaques. The right coronary artery, at a point 6 cm. from its origin, was almost completely stenosed by a plaque into which massive hemorrhage had occurred. The aorta was also markedly atherosclerotic and showed numerous points of intimal erosion. Underlying an erosion in the abdominal aorta was a large intramural hematoma, measuring 4 cm. in diameter. The main branches of the cerebral arteries showed gross evidence of athero-

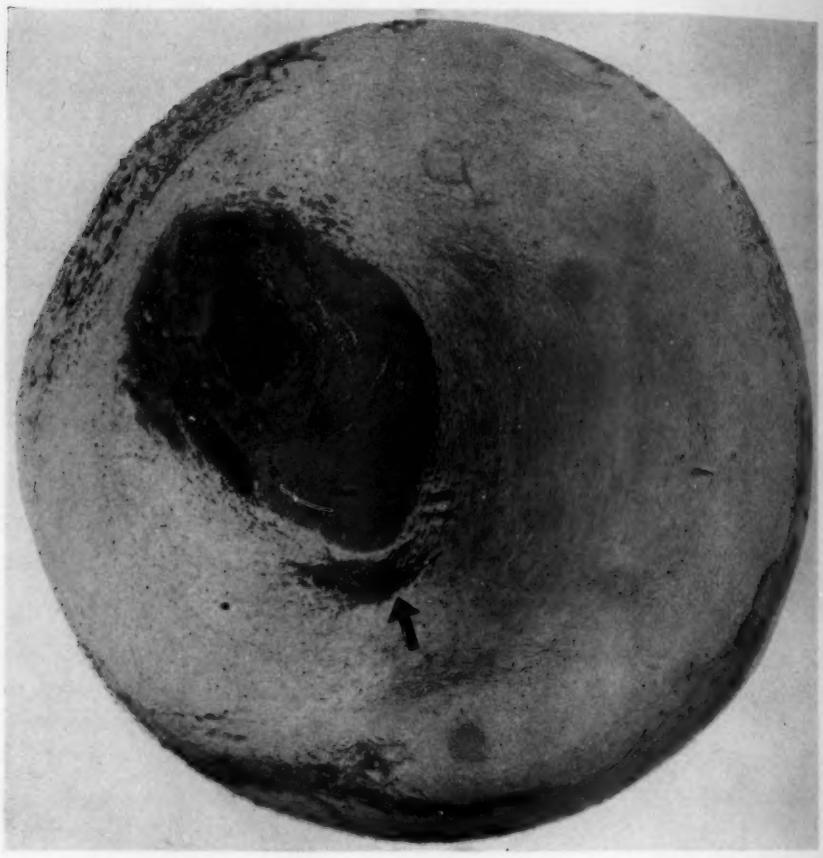


Fig. 2.—A low power photomicrograph of a thrombosed cerebral artery. A small intimal hemorrhage (shown by the arrow) lies close to the oldest part of the thrombus. Hematoxylin and eosin; × 20.

sclerosis with numerous points of stenosis. Projecting from the outer surface of the left posterior cerebral artery was a reddish brown rounded nipple-like mass, measuring 3 mm. in diameter. Two similar hemorrhagic lesions were noted on the outer surface of the right posterior cerebral artery. These 3 hemorrhages were embedded separately and sectioned serially. The remaining portions of the principal cerebral arteries were cut into short segments, embedded in bundles, and sectioned serially at intervals of 200 microns.

Microscopically, 12 intimal hemorrhages, including the 3 noted on gross examination, were found in various parts of the principal cerebral arteries. Each hemorrhage had occurred into an area of atheromatous degeneration. Occasionally the lumen appeared to be stenosed by the hemorrhage. Many of the intimal hemorhages lay in the outer zones of the thickened intima, in contact with the media (fig. 1 *A*). The hemorrhages varied in age; some consisted of intact red cells, while in others the red cells had disintegrated, and stainable iron was present. One artery with an outside diameter of 2.5 mm. showed almost complete stenosis of the lumen by atherosclerotic thickening of the intima, recent hemorrhage into the outer zone of the intima and complete occlusion of the lumen by thrombus material of fairly recent formation. Capillaries lay in proximity to several of the intimal hemorrhages, and in 1 instance serial section showed one of these capillaries to arise from the lumen of the artery. Sections through the intimal hemorrhage in the right coronary artery showed marked stenosis of the lumen by an atheromatous plaque in which there was a large amount of hemorrhage, consisting of disintegrating red cells and yellowish pigment. The hemorrhage was so massive that it had obviously compressed the already stenosed coronary lumen. Sections through the intramural hematoma of the abdominal aorta showed the hemorrhage to be confined to a large intimal atheroma. Numbers of capillaries lay between it and the endothelial lining.

Six cases of cerebral thrombosis have been studied to date. The thrombosed segments of the arteries were sectioned serially throughout their length. Intimal hemorrhage was found at the point of thrombus precipitation in 4 of the 6 cases. When parts of a single thrombus varied in age (as determined by the amount of organization), the oldest part was attached to the arterial wall close to an intimal hemorrhage (fig. 2). Both the thrombi and the intimal hemorrhages were of recent origin in 3 of the 4 cases, while in 1 case the thrombus was organized and the intimal hemorrhage largely converted into pigment. In this case there was also an organized thrombus with an old intimal hemorrhage in the right coronary artery, as well as a number of fresh intimal hemorrhages in both branches of the left coronary artery. Each of the cerebral thrombi which were associated with intimal hemorrhages had formed at a point of stenosis of the lumen of the artery. Capillaries lay in proximity to the intimal hemorrhages and to the arterial lumens in several cases.

Finally, a case has been observed in which an intimal hemorrhage had ruptured through the medial coat of the artery and leaked into and through the adventitial fibers (fig. 3). A summary of the history and the observations at autopsy in this case follows:

Case 2.—A 72 year old man gave a history of senile mental deficiency, poor memory and inability to move the left arm for many years. Just before admisson swelling of the feet and ankles developed, with breathlessness on the slightest exertion. The blood pressure on admission was 150 systolic and 90 diastolic, but later it rose to 184 systolic and 100 diastolic. Signs of bilateral bronchopneumonia developed and the patient died about two weeks after admission.

The principal observations at autopsy were confined to the brain and meninges. The dura mater covering the inner surface of the convexity of the left cerebral hemisphere was thickened up to 2 mm. by a deposit of orange-brown old blood pigment covered by a smooth shining membrane. The large arteries at the base of the brain showed marked atherosclerosis. The left middle cerebral artery at a point just beneath the tip of the temporal lobe was markedly sclerotic over a distance of about 1 cm., and over most of this area the wall of the vessel was

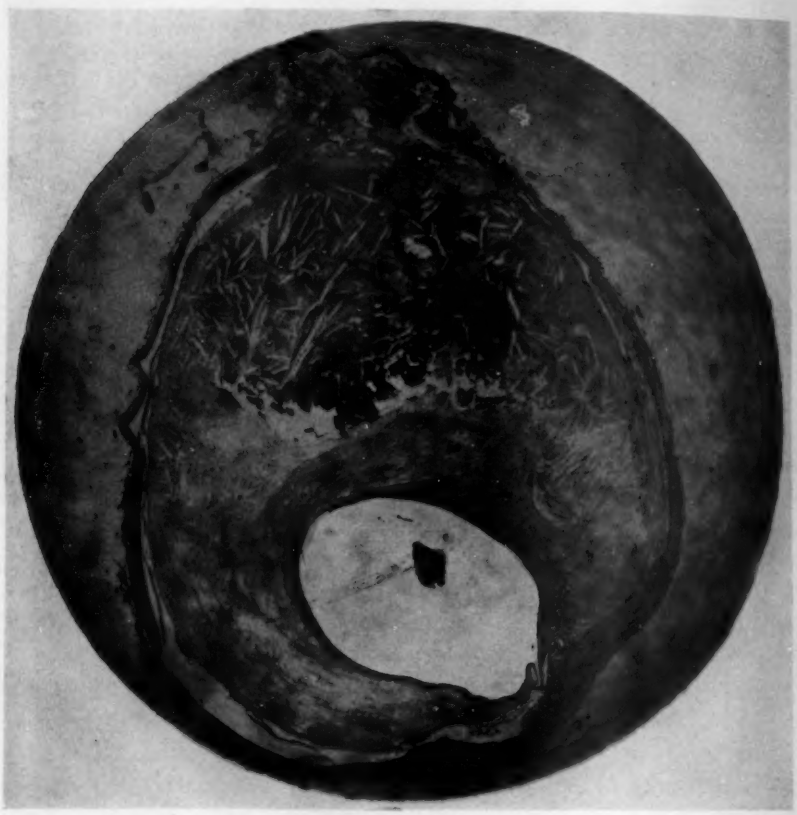


Fig. 3.—Photomicrograph of a cerebral artery in case 2 in which a large intimal hematoma has broken through the media and involves the adventitial coat. Hematoxylin and eosin; × 18.

distended. In one segment of the sclerotic patch there was a nipple-like projection of the adventitial coat, the outer layers of which were stained with old blood pigment. Examination of the brain tissue revealed bilateral internal hydrocephalus of the lateral ventricles, and a cystic defect, measuring 1 cm. in diameter, of the white matter overlying the roof of the posterior horn of the right lateral ventricle. One half of the nipple-like projection of the left middle cerebral artery was sectioned serially at intervals of 8 microns.

Microscopically, the projecting mass on the left middle cerebral artery was made up of a large hematoma consisting of both fresh and old hemorrhage. The hemorrhage lay for the most part in the outer half of a large atheromatous plaque of the intima, but in some of the sections it had broken through the media and lay among the adventitial fibers (fig. 3). A thick layer of dense fibrous tissue separated the intimal hemorrhage from the lumen of the artery. No intimal capillaries were noted in the sections studied.

COMMENT

Intimal hemorrhages in sclerotic cerebral arteries are identical in structure with those described previously in atherosclerotic plaques of human coronary arteries. They occur only in atheromatous foci, and they are intimately related to capillaries which arise from the arterial lumens. It is generally admitted, now, that intimal hemorrhages of the coronary arteries are intrinsic lesions resulting from the rupture of capillaries which are derived from the lumens of the main arteries. It may be assumed that intimal hemorrhages of the cerebral arteries are produced in a similar manner. This assumption is supported by the fact that coronary and cerebral intimal hemorrhages are not infrequently found in the same patient, a fact which suggests a common etiologic agent.

Likewise, the precipitating factor of thrombosis in sclerotic cerebral arteries appears to be the same as that in thrombosis of the coronary arteries. I have described elsewhere [1] the common finding of intimal hemorrhages at the points of precipitation of coronary thrombi, and this has been confirmed by others.[5] To date, 47 cases of coronary thrombosis have been studied by fairly exhaustive methods, including serial sections in many instances, and in 41 cases intimal hemorrhage was found at the site of precipitation of the thrombus. The common association of intimal hemorrhages and coronary thrombi in a series as large as this and the confirmatory evidence of others eliminate the possibility of this association being coincidence. Because intimal hemorrhage often occurs without thrombosis of the adjacent arterial lumen, such hemorrhages cannot be regarded as resulting from the presence of the thrombi. One is forced to the conclusion, therefore, that most coronary thrombi are precipitated either by the intimal hemorrhages proper or by other lesions that result from the rupture of intimal capillaries. The same conclusion must be reached in regard to thrombi in cerebral arteries. Although my series is small the intimal hemorrhages found at the sites of precipitation of 4 of 6 cerebral thrombi were identical in all respects with those observed in coronary arteries.

A certain amount of evidence has been collected to show that other cerebral vascular lesions besides thrombosis may be related to intimal

5. Finkelstein, L., and Horn, H.: Personal communication to the author.

hemorrhages. Of particular interest is their relation to arteriospastic attacks in hypertensive persons as suggested in case 1 in this series. The clinical signs of cerebral arteriospastic attacks over a period of four years in this case and the postmortem observation of 12 distinct intimal hemorrhages of varying ages in the larger cerebral arteries suggest that they were related. Many of the hemorrhages lay at the outer borders of atheromatous plaques in proximity to the media (fig. 1 *A*), and it is reasonable to suppose that the sudden disruption of tissue by the hemorrhages may have set up transient spasms of the muscle coat. The observations of others tend to confirm this hypothesis. The walls of cerebral arteries are known to be supplied by vasomotor fibers, and local spasm due to local injuries or influences definitely occurs. Stroking the adventitia of a pial artery with a blunt instrument at operation causes spasm of that part of the vessel.[6] Also, injuries to arterial walls are known to cause pain. Waterston [7] found that the contact of the point of a needle with the wall of an artery elicited sharp pain, and when the needle point was pushed into the wall a peculiar sickening pain, associated with nausea and faintness, resulted. Feiling,[8] and Aring and Merritt [9] commented on the frequency with which certain prodromal symptoms occur in patients with cerebral arterial thrombosis. The symptoms consist of headache, dizziness, transient weakness of one or both limbs or of one side of the face, temporary aphasia and other symptoms. Sometimes slight hemiplegic weakness may appear and rapidly vanish, to be followed a few days later by severe hemiplegia. It is possible that these premonitory symptoms in certain cases, including pain in the head, are due to arteriospasm from irritation by the intimal hemorrhages which precede and apparently cause cerebral thrombosis. It must be admitted, however, that attempts to demonstrate nerve fibers in the actual area of intimal hemorrhage have as yet been unsuccessful.

Equally hypothetic is the relation of intimal hemorrhage to cerebral arterial rupture. An accidental finding in a case in my series was a large intimal hematoma of a middle cerebral artery which had broken through the thin medial layer and lay between the adventitial fibers (fig. 3). It is possible that a true intracranial hemorrhage would have occurred in this case if the process had continued. Cases of classic cerebral hemorrhage due to rupture of the lenticulostriate artery have not as yet been studied, but the observations of others suggest that in this vessel also the precursor of rupture may be an intimal hematoma.

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6. Penfield, W.: Personal communication to the author.
7. Waterston, D.: Lancet **1**:943, 1933.
8. Feiling, A.: Practitioner **133**:62, 1934.
9. Aring, C. D., and Merritt, H. H.: Arch. Int. Med. **56**:435, 1935.

Ellis [10] found that the miliary aneurysms which are usually regarded as the cause of cerebral hemorrhage are not true aneurysms but are encapsulated hematomas. This important question is now being studied and will be discussed later.

From the observations in this short series of cases it appears that capillary rupture with intimal hemorrhage is intimately concerned with the mechanism of cerebral arterial thrombosis and possibly with the causation of cerebral arteriospasm and hemorrhage in certain cases. If this is true, the factors responsible for the rupture of intimal capillaries should be the immediate causes of these lesions. The factors responsible for the rupture of intimal capillaries in the coronary arteries have been described elsewhere,[1] and the same factors hold good for the cerebral vessels. The integrity of a capillary wall in any part of the body depends on the pressure of blood within its lumen, the strength and elasticity of its wall, and the rigidity of its supporting stroma. Because intimal capillaries arise directly from the lumen of a large artery, it is argued that they will be subjected to a greater blood pressure than those which lie at the end of a long series of arteries and arterioles. This argument is not new: Ellis [10] stated that "the small vessels of the basal ganglia, notoriously the site of hemorrhage, are direct branches of the cerebral arteries and hence are subjected to a blood pressure greater than vessels of similar size in other parts of the brain and possibly of the body." It is reasonable to assume that the pressure in intimal capillaries of the cerebral arteries, normally high, will be increased in cases of persistent hypertension or of temporary elevation of blood pressure from excessive exercise or emotion. In these circumstances the strain on the capillary walls will be increased, and there will be imminent danger of capillary dilatation and rupture. In the series reported here, persistent hypertension was present in 3 of the 4 cases in which intimal hemorrhage was observed at the site of precipitation of a thrombus. Aring and Merritt [9] investigated 96 cases of cerebral thrombosis and found that in 85 per cent the systolic pressure was in excess of 140 mm. of mercury and that in 50 per cent the diastolic pressure was in excess of 100 mm. of mercury.

Loss of rigidity of the tissues supporting the capillary walls appears to be equally important in the causation of capillary rupture in cerebral arteries. Each of the intimal hemorrhages in this series was found in an area of atheromatous degeneration; the softening process which is characteristic of atheroma allows the capillaries to dilate, and if the intracapillary pressure is high enough, overdilatation and rupture may occur. Theoretically, replacement of the atheromatous material by

10. Ellis, A. G.: Publ. Jefferson M. Coll. & Hosp. **5**:1, 1915.

calcific deposits should help to strengthen the supporting stroma and prevent capillary dilatation and rupture, but it is noteworthy that calcification in the cerebral arteries is rare, and none at all was seen in this series.

Finally, the strength and elasticity of the walls of capillaries in cerebral arteries may be affected by a number of factors. It is known that capillary fragility increases with advancing age, vitamin deficiency or local inflammatory conditions. Each of these factors may be of importance in the production of intimal hemorrhage in certain cases.

SUMMARY

Intimal hemorrhages in sclerotic cerebral arteries are similar in structure to those described previously in sclerotic coronary arteries. They result, not from backflow of blood through defects produced by rupture of atheromatous "abscesses," as was previously thought, but from rupture of capillaries derived from the main arterial lumens.

From the observations in this series it appears that capillary rupture with intimal hemorrhage is intimately concerned with the mechanism of cerebral arterial thrombosis and possibly, in certain cases, with the causation of cerebral arteriospasm and rupture. It is suggested that the factors responsible for the rupture of intimal capillaries in the cerebral arteries are high intracapillary pressure from hypertension, progressive atheromatous degeneration of the supporting tissues and increased capillary fragility from a variety of causes.

EFFECTS OF PROLONGED INJECTIONS OF BOVINE ANTERIOR PITUITARY EXTRACT ON BONE AND CARTILAGE OF GUINEA PIGS

MARTIN SILBERBERG, M.D.
AND
RUTH SILBERBERG, M.D.
ST. LOUIS

In immature guinea pigs injections of an acid extract of the anterior lobe of the bovine pituitary for periods of from four to twenty-one days promoted the formation of cartilage and bone.[1] The course of endochondral ossification was determined by the rate at which the proliferation of the cartilage and its subsequent replacement by bone took place.[2] In the majority of cases the calcification and ossification of the cartilage predominated over the new formation of cartilage cells and this led to narrowing of the epiphysial disk. In other instances, however, the proliferation of cartilage was more stimulated than ossification and the epiphysial lines, therefore, remained open. In order to determine whether these reactions represented a temporary condition or changes of a more permanent nature, we investigated the alterations in the skeletal tissues after administration of the anterior pituitary extract for longer periods of time.

MATERIAL AND METHODS

Thirty-seven guinea pigs of both sexes, born at different seasons and with an initial weight of from 135 to 175 Gm., were used. Each of eighteen guinea pigs received a daily intraperitoneal injection of 1 cc. of a freshly prepared acid extract of the anterior lobe of the bovine pituitary for from one to six months, while 19 guinea pigs served as controls.

Of a total of 18 treated guinea pigs, 5 received injections for one month, 5 for two months, 2 for three months, 2 for four months and 1 for six months. Two additional guinea pigs which had been given injections for three months and another which had been treated for four months were allowed to survive for an additional period of four months, during which no injections were given.

As to the technical procedure of removing the bones and preparing the specimens, we refer to our previous reports.[2]

From the Laboratory of Research Pathology, Oscar Johnson Institute, Washington University School of Medicine.

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1. Silberberg, M.: Proc. Soc. Exper. Biol. & Med. **32**:1423, 1935.
2. Silberberg, M., and Silberberg, R.: Arch. Path. **26**:1208, 1938.

GENERAL OBSERVATIONS

The mean weights of the guinea pigs are given in table 1.

At early stages the gain in weight in the treated guinea pigs was less than in the control animals. At later stages, in the majority of the treated animals, even in those which had, for a while, shown a dwarfed appearance, a considerable gain in

TABLE 1.—*Mean Weights and Deviations from the Means of Normal and Treated Guinea Pigs.*

	Total Number	Initial Weight, Gm.	Weights After					
			1 Mo.	2 Mo.	3 Mo.	4 Mo.	5 Mo.	6 Mo.
Normal animals	19	(19 animals) 140 +15 −10	(19 animals) 242 +63 −57	(15 animals) 341 +89 −96	(11 animals) 431 +129 −106	(8 animals) 520 +40 −80	(6 animals) 600 +116 −40	(1 animal) 640 +0 −0
Treated animals	18	(18 animals) 140 +26 −14	(18 animals) 194 +36 −64	(13 animals) 279 +101 −139	(8 animals) 352 +108 −122	(4 animals) 438 +47 −103	(1 animal) 585 +0 −0	(1 animal) 615 +0 −0

TABLE 2.—*Individual Weights of Two Pairs of Guinea Pigs Which Had Been Given Injections for Two and Three Months, One Pair Having Been Allowed to Survive After Discontinuation of the Injections*

	Guinea Pig 170/114			Guinea Pig 171/114			Guinea Pig 43			Guinea Pig 44		
Initial weight, Gm.	185			180			140			145		
1 week*	**175**	**175**	**165**	**175**	**175**	**175**	**150**	**140**	160	**145**	**125**	**140**
2 weeks	**180**	**180**	**185**	**180**	**185**	**180**	155	160	175	**140**	**155**	**140**
3 weeks	190	210	200	**180**	200	195	**160**	**145**	**150**	**130**	**125**	**145**
4 weeks	205	230	**215**	205	225	210	**170**	**175**	**160**	**140**	**140**	**135**
5 weeks	**220**	**230**	**230**	220	245	255	**155**	**155**	**160**	**125**	**130**	**135**
6 weeks	**195**	**235**	235	200	260	265	180	**170**	**180**	**145**	**140**	160
7 weeks	250	235	265	290	290	325	205	210	**200**	165	170	175
8 weeks	260	265	275	320	345	380	**205**	**190**	**200**	180	**165**	**180**
9 weeks	.....	.....	.....	.....	.....	.....	**190**	**160**	**180**	**190**	**175**	190
10 weeks	.....	.....	.....	.....	.....	.....	**175**	**180**	**190**	195	200	215
11 weeks	.....	.....	.....	.....	.....	.....	**185**	**155**	**195**	**205**	**175**	**220**
12 weeks	.....	.....	.....	.....	.....	.....	**180**	**170**	**160**	**205**	**210**	**215**
13 weeks	.....	.....	.....	.....	.....	.....	**200**	**195**	230	230	205	230
Injections discontinued												
14 weeks	.....	.....	.....	.....	.....	.....		315			340	
15 weeks	.....	.....	.....	.....	.....	.....		330			350	
16 weeks	.....	.....	.....	.....	.....	.....		350			360	
17 weeks	.....	.....	.....	.....	.....	.....		370			350	
18 weeks	.....	.....	.....	.....	.....	.....		420			405	
19 weeks	.....	.....	.....	.....	.....	.....		385			370	
20 weeks	.....	.....	.....	.....	.....	.....		400			390	
Onset of pregnancies												

* The bold face numbers mark either a lack of gain or a loss in weight.

weight and comparatively fast growth were seen; after three months the animals could hardly be distinguished from normal controls. This agrees with the finding of Loeb[3] that a marked decrease in hyperactivity of the thyroid gland takes place step by step in the course of prolonged injections of anterior pituitary extract.

The individual weights of two pairs of guinea pigs are given in table 2. Each pair had been treated at the same time and with the same dose of the same extract;

3. (*a*) Loeb, L., and Basset, R. B.: Proc. Soc. Exper. Biol. & Med. **26**:860, 1929. (*b*) Loeb, L., and Friedman, H.: ibid. **29**:172, 1931.

nevertheless, the weight curves differed, one rising steadily after an initial period of arrest of weight, and the other showing a second period of cessation of weight increase, following a period of growth. The weights of these animals seem of interest with reference to the question whether periods of cessation in gain or periods of loss of weight may be correlated with so-called lines of arrested growth in bone.

In table 2 we have marked periods of lack of gain in weight or loss in weight with asterisks.

Measurements of the length of the tibia and femur were taken by means of a caliper. In regard to these we found, however, even in normal animals, considerable variations, amounting to as much as 10 per cent and more, between different guinea pigs. For instance, with normal guinea pigs weighing 180 to 190 Gm. it was found that the length of the tibia was 3.5 cm. in some and only 3.1 cm. in others. Similar variations were found in the treated animals. In view of these variations, and because we did not use standardized strains of animals in our experiments, we did not think it feasible to draw definite conclusions from the measurements of the bones; instead, we relied on the histologic study of these tissues.

MICROSCOPIC OBSERVATIONS

As to the normal microscopic structure of the bone and cartilage of the guinea pig at different ages, we refer to former publications.[4]

After one month's treatment with the anterior pituitary extract the cartilage and bone at the upper epiphysial zone of the tibia were seen to react in one of two ways:

1. In 3 of 5 animals which had lost or which had not gained weight the zone of endochondral ossification was distinctly narrowed. The intercartilaginous ground substance was acidophilic, sclerosed, and resembled preosseous substance. Instead of ten columnar cells as observed ordinarily, only five to six, or even fewer, cells were counted in the epiphysial cartilage row; instead of the normal number of four hypertrophic cells in one row, only two cells or a single one was found. Sometimes whole rows of cartilage cells had undergone retrogression; such circumscribed areas were filled in with wedgelike osseous plugs. In general, an intensified calcification of the cells of the hypertrophic layer was associated with or followed by rapid ossification, either by breakdown of cartilage cells following ingrowth of capillaries into the cartilage and subsequent replacement by bone or, here and there, by direct conversion of hypertrophic cartilage cells into osteocytes. In the subepiphysial layer the trabeculae were numerous and thickened, and their main axes were arranged in a transverse as well as in a longitudinal direction; if, however, fibrosis of the marrow was found, the trabeculae were thinned out, contained much calcium and were arranged in a longitudinal direction or were destroyed by the proliferating connective tissue of the bone marrow. In the chondrophyte the intercartilaginous matrix was likewise sclerosed, some cartilage cells were hyperplastic, and hypertrophic incubator capsules appeared, which in places underwent retrogression. The cartilage of the joint was thickened; the cartilage cells, particularly those of the transitional and pressure zones, proliferated by way of amitosis; they took on a perpendicular arrangement, and four and more cells were surrounded by a common capsule. Some of these cells underwent hypertrophy followed by karyorrhexis and karyolysis. The bony border lamella in some areas was corroded by capillaries

4. Silberberg, M., and Silberberg, R.: Am. J. Path. (*a*) **15**:547, 1939; (*b*) **15**:55, 1939; footnote 2.

advancing from the bone marrow of the epiphysis; however, calcification and ossification also progressed. In ribs and vertebrae the state of cartilage and bone was similar to that in the long bones.

2. In 2 of 5 guinea pigs the findings were different; here, the epiphysial lines were patent and wider than under corresponding normal conditions. The cartilaginous matrix of the epiphysial line was loosened and swollen. Not only single cells, but not infrequently whole rows of cartilage cells, degenerated and disintegrated. But, on the other hand, proliferation of the resting and especially of the columnar cartilage cells had also taken place occasionally by way of mitoses. Instead of the ordinary number of ten columnar cartilage cells situated in one row, as many as twenty and more cells were counted, whereas the number of hypertrophic cartilage cells was apparently unchanged. In those instances in which there was predominance of proliferation of the cartilage over ossification, the trabeculae were not thickened. Here also a certain tendency of the bone marrow to undergo fibrosis was noticeable. In the joint the proliferation of the cartilage cells, in association with intensified processes of retrogression and solution, had led to the development of minute arthropathic lesions, which were more accentuated here than in the previous cases in which ossification predominated.

After two months of injections of the extract these two different modes of reaction were still present. In 3 of 5 animals the epiphysial line was distinctly narrowed (fig. 1 *B*), and ossification of the cartilage was progressing. These 3 animals had gained only a little weight. The sclerosed cartilaginous ground substance had become more osseous, and the fibrils were thickened. The cartilage cell columns were irregular; in some areas replacement of cartilaginous tissue by bony material had occurred, which in places had led to the formation of osseous bridges between the epiphysis and the diaphysis (fig. 2 *A*). Besides such bony bridges, wedgelike osseous plugs of varying length and thickness were seen. Not all these bony structures which had replaced the disintegrated cartilage cells persisted unchanged. Some of them were corroded and absorbed by constituents of the bone marrow advancing from the metaphysis; others underwent processes of solution by preserved neighboring cartilage cells, which continued to proliferate. The covering of the joint was thickened owing to the hyperplasia and increased ossification of the cartilage. In the 2 other animals, which had gained more weight, the epiphysial line was still of medium width, and proliferation of the cartilage cells was more accentuated than ossification of the cartilage. However, in contradistinction to the loosening and swelling of the matrix as observed after one month's injections of the extract, sclerosis of the cartilaginous ground substance and its fibrils was seen. In the joints there was thickening of the covering caused by intensive proliferation of the cartilage cells associated with processes of retrogression, solution and vascularization. In the bone marrow, ribs and vertebrae the changes were essentially the same as those found after one month.

After three or more months of treatment the two main types of reaction could still be recognized: Either the epiphysial line had become more and more ossified, and more osseous plugs appeared, connecting the trabeculae of the diaphysis with the epiphysis, while processes of solution of bone by cartilage cells and bone marrow could also be observed, or proliferation of cartilage cells was still proceeding, although at these later stages the individual cartilage cells were rather flat, the sclerosis of the cartilaginous matrix had increased, and ossification had set in in various places (fig. 2 *B*). This twofold mode of reaction of the cartilage was reflected in the behavior of the bony tissues. Predominance of proliferation of the cartilage cells was associated with increased absorption and solution of bone.

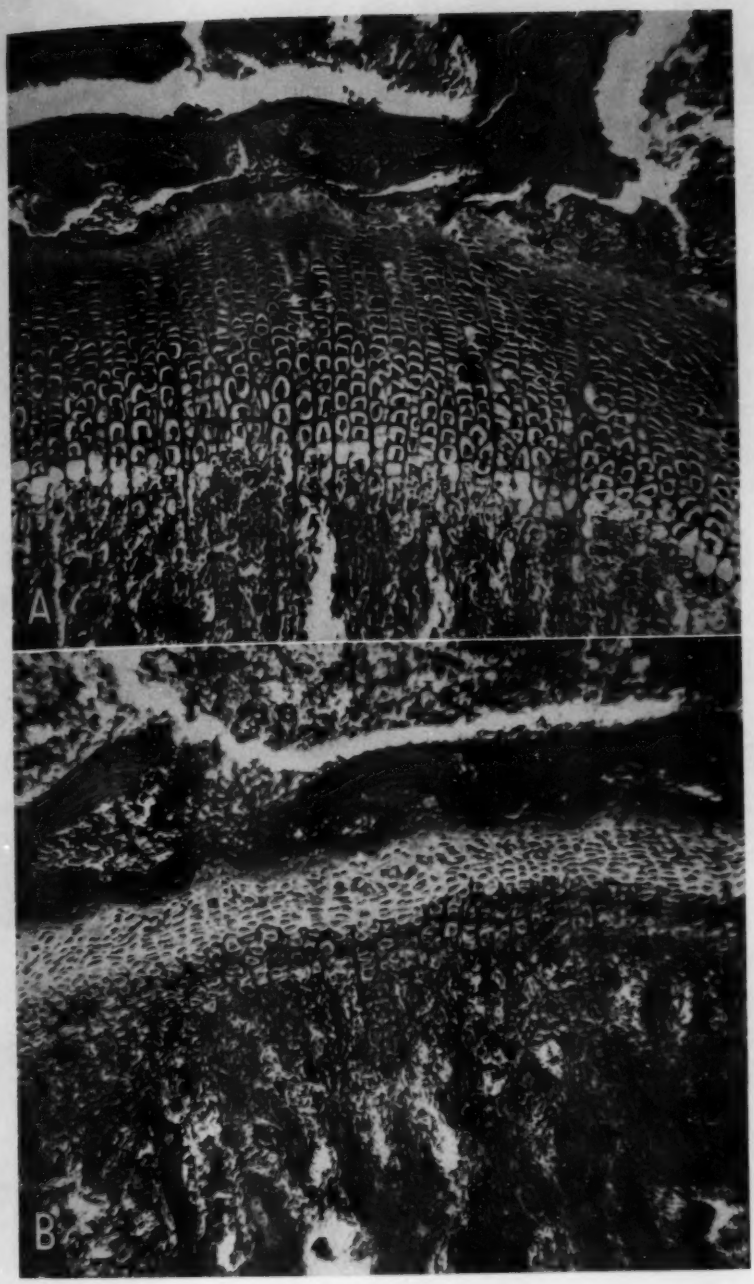


Fig. 1.—*A*, section through the upper part of a tibia of a normal female guinea pig weighing 275 Gm. and of the same age as guinea pig 38. The epiphysial line is of medium width; the cartilage rows are regularly arranged; there is no evidence of changes in the cartilaginous ground substance. Magnification, 150. *B*, section through the upper part of a tibia of a female guinea pig (38) which had shown an initial weight of 150 Gm. and which received injections of 1 cc. of anterior pituitary extract for two months. The final weight was 175 Gm. The epiphysial line is narrowed; the columnar cartilage cells are diminished in number; a dense calcified layer of hypertrophic cartilage and thick bony trabeculae are seen. Magnification, 150.

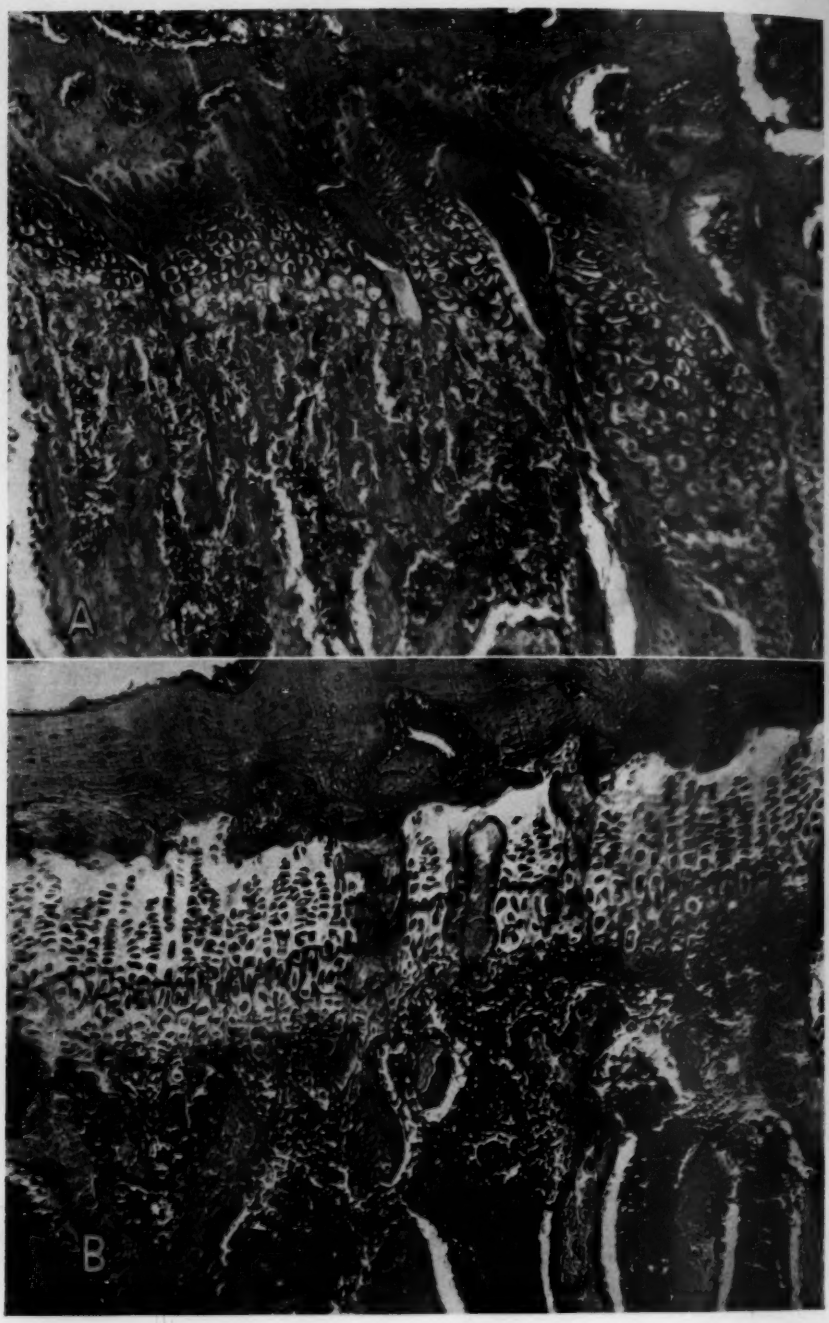


Fig. 2.—*A*, section through the upper part of a tibia of a female guinea pig (170/114) which had shown an initial weight of 175 Gm. and which had been given injections of 1 cc. of anterior pituitary extract daily for two months. The final weight was 275 Gm. The epiphysial line is narrowed; four bony plugs have replaced destroyed cartilage cell rows; one plug is being invaded and dissolved by bone marrow. Magnification, 150. *B*, section through the upper part of the tibia of a female guinea pig (44) which weighed initially 145 Gm. and which had been given injections of 1 cc. of anterior pituitary extract daily for three months; subsequently the animal lived for four additional months. The final weight was 550 Gm. The epiphysial line is of medium width; the cartilage cells are fairly numerous but irregularly arranged; three thick osseous plugs are seen traversing the epiphysial disk and replacing cartilage cell rows; the matrix is sclerosed; the trabeculae are thickened. Magnification, 150.

This condition manifested itself in thinning and corrosion of the osseous border lamella of the joint as well as in the slender configuration of the bony trabeculae. Increased ossification of the epiphysial zone, on the other hand, was associated with deposition of more osseous substance in the trabeculae of the marrow, in the compacta of the shaft and in the covering of the joint. The thicker the compact bone of the shaft, the less numerous were the haversian canals and, correspondingly, the less marked were the resorptive processes which were observed.

In an animal which had been allowed to survive for four months after discontinuation of the injections there appeared three lines of transverse trabeculae parallel to the zone of ossification (fig. 3). Between these trabeculae and the epiphysial line one could detect islands of nonossified mature euhyaline cartilage.

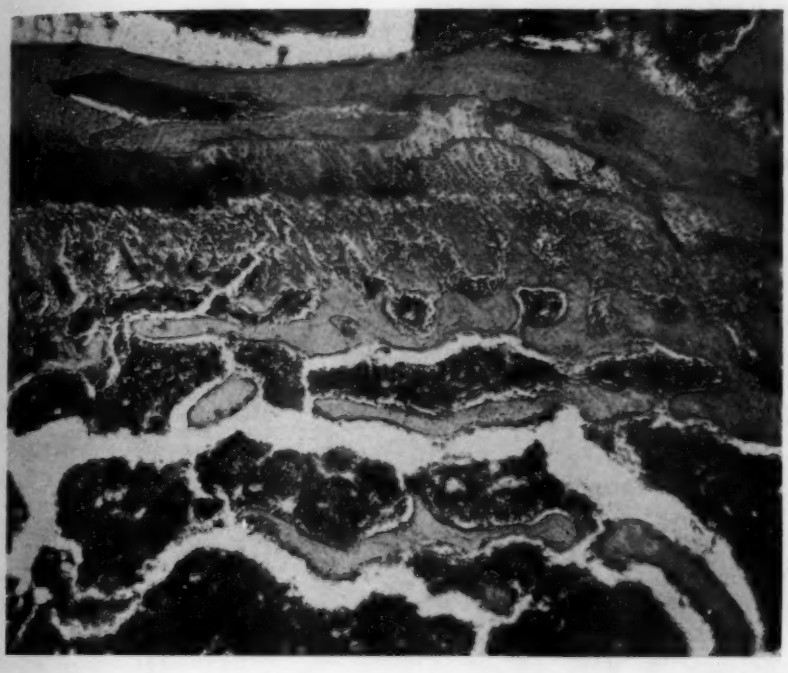


Fig. 3.—Section through the upper part of a tibia of a female guinea pig (43) with an initial weight of 140 Gm. (same experiment as in fig. 2 *B*). The final weight was 535 Gm. The epiphysial line is fairly cellular and partly calcified. Note the presence of three lines of trabeculae with axes arranged in a transverse direction. Magnification, 120.

Table 3 gives concise descriptions of the changes in the weights of the guinea pigs and brief statements concerning the presence of so-called lines of arrested growth in their bones; furthermore, an attempt is made there to correlate the presence of lines of arrested growth [5] in the histologic section with periods of cessation of gain or of loss in weight.

5. Harris, H. A.: Bone Growth in Health and Disease, New York, Oxford University Press, 1933.

TABLE 3.—*Summary of Observations on Relationship Between Cessation of Weight Increase and Lines of Arrested Growth in Bones*

Animal	Experiment	Histologic Observations as to the Presence of Lines of Arrested Growth	Weights
59	Extract for 6 mo.	Some transverse trabeculae	Two definite periods of standstill: 1st period, lasting 3 weeks, began 11 days after treatment was started, during which time gain in weight was good; 2d period, lasting 4 weeks, during 3d and 4th months of experiment
59/119 (239A)	Extract for 4 mo.	No line of arrested growth	Initial period of standstill lasting 12 days
64/119 (328A)	Extract for 4 mo.	Few transverse trabeculae at some distance from the epiphysial line	Initial period of cessation of growth lasting 2½ weeks
64	Extract for 3 mo.	No line of arrested growth	Initial standstill lasting 2 weeks, then slow growth up to the end of the 2d month, more rapid growth during 3d month
88/119 (110A)	Extract for 3 mo.	No line of arrested growth	Initial period of standstill lasting 2 weeks; 2d period of standstill lasting 1 week at end of 2d month
67	Extract for 2 mo.	No line of arrested growth	Initial standstill lasting 1 week, then slow gain during 1st month; more rapid gain during 2d month
38	Extract for 2 mo.	Transverse bony trabeculae directly adjoining the hypertrophic cartilage cell layer	Initial period of standstill lasting 3 weeks; 2d period lasting 3 weeks during 2d month
74/119	Extract for 2 mo.	Transverse trabeculae in metaphysis	No cessation of growth
170/119 (115A)	Extract for 2 mo.	Transverse trabeculae at some distance from the epiphysial line	Initial period of standstill lasting 2 weeks; 2d period lasting 1 week during 1st half of 2d month
171/114 (116A)	Extract for 2 mo.	No line of arrested growth	Initial period of standstill lasting 2½ weeks
58	Extract for 1 mo.	No line of arrested growth	Almost complete cessation of weight increase during time of experiment except for gain during 3 to 4 days
73	Extract for 1 mo.	No line of arrested growth	Complete standstill for the initial period of 10 days, then slow growth
74	Extract for 1 mo.	No line of arrested growth	Complete arrest for an initial period of 5 to 6 days, then good growth
143/114 (300A)	Extract for 1 mo.	Few transverse trabeculae adjoining epiphysial line in the center	Initial period of standstill lasting 2 weeks
86/119 (137A)	Extract for 1 mo.	No line of arrested growth	Initial period of standstill lasting 2 weeks
40	Extract for 4 mo., followed by 4 mo. without injections	No line of arrested growth; marked ossification	Very slow gain in weight but no definite standstill during the 1st month, except for the 1st 4 days
43	Extract for 4 mo., followed by 4 mo. without injections	Three definite transverse osseous lines	Three definite periods of standstill, 1st one at beginning of experiment lasting 4 days, a 2d one lasting 2 to 3 weeks during 2d half of the 1st month and 1st half of the 2d month, and a 3d lasting 4 weeks during the 4th month, followed by a rapid gain
44	Extract for 3 mo., followed by 4 mo. without injections	No line of arrested growth; very marked ossification	Initial period of standstill lasting 5 to 6 weeks; 2d period lasting 2 weeks during the 4th month. At this time weight almost identical with that of guinea pig 43; after discontinuation of injections rapid increase in weight

With the exception of 1 animal (74/119) all guinea pigs showed a period of cessation of weight increase immediately after the beginning of the injections. This period varied from four days to six weeks. In some cases, after this period of standstill, there occurred a continuous, if rather slow, gain in weight. In others (43 and 44) a slight gain in weight of about 15 Gm. took place over a period of several days, followed by another period of standstill.

As to the parallelism between the cessation of gain in weight and the occurrence of lines of arrested growth in the microscopic sections, 3 cases are of particular interest. Although guinea pig 74/119 did not suffer any cessation of weight increase except for an early drop of 20 Gm. within two days, still in the diaphysial marrow, instead of the ordinary longitudinal arrangement the axes of the trabeculae were definitely arranged in a transverse direction. On the other hand, guinea pig 44, in which cessation of gain in weight was most pronounced, did not reveal any evidence of such a line; on the contrary, it showed a very even distribution of osseous trabeculae, with their axes arranged in a longitudinal direction. Guinea pig 43 was the only animal in which three periods of cessation of gain in weight were accompanied by three definite transverse lines of osseous trabeculae in the marrow cavity.

Our findings are summarized in table 4.

TABLE 4.—*Relationship Between Behavior of Weight and Lines of Arrested Growth in Treated Guinea Pigs*

Guinea Pigs	Number with Initial Loss of Weight	Number with Secondary Lack of Gain in Weight	Number with No Cessation of Gain in Weight	Number Showing Lines of Arrested Growth		Number Showing no Lines of Arrested Growth
				Pronounced	Trace	
18	16	6	2	1	6	11

From this summary it may be concluded that the occurrence of transverse trabeculae in the marrow cavities of guinea pigs under certain experimental conditions is not definitely correlated with the cessation of gain in weight in these animals.

COMMENT

The results obtained after prolonged administration of the acid extract of the anterior lobe of the bovine pituitary supplement and can be correlated with our findings in bone and cartilage after injections of this substance for shorter periods of time.[6]

Two types of reaction can be distinguished. The one is characterized by a predominating stimulation of the formation of cartilage, with com-

6. With reference to a recent publication by J. Freud, L. H. Levie and D. B. Kroon (J. Endocrinol. **1**:56, 1939) it may be mentioned that the extract used by us had been prepared according to the method of L. Loeb and R. B. Basset (Proc. Soc. Exper. Biol. & Med. **26**:860, 1929), as stated in our previous reports (Silberberg, M.: Virchows Arch. f. path. Anat. **289**:201, 1933; footnote 1). Freud, Levie and Kroon stated, furthermore, that only limited histologic data are available. In several of our previous papers (footnotes 1 and 4 *b*) we gave full histologic descriptions of our observations, which were illustrated by photomicrographs. Their paper is essentially confirmatory of our findings.

paratively little degeneration and ossification, and wide epiphysial zones, rich in cartilage cells. The second response consists of a proliferation of cartilage cells, associated with extensive degeneration in places and early replacement of the degenerated areas by bone. In the latter case the increased and premature ossification leads to narrowing and partial closure of the epiphysial disk.

The tendency of the extract to promote ossification finds expression also in thickening of the trabeculae, of the osseous parts of the covering of the joints and of the compact cortex of the long bones. This tendency, however, becomes apparent only at later periods. At earlier stages it is interfered with by increased resorption of osseous material, presumably under the influence of the hyperactivity of the thyroid gland which has been called forth by the anterior pituitary extract.

It was at first assumed by us that the disturbance of the balance between the new formation of cartilage and its ossification was due to differences in the degree of stimulation of these two processes. A higher degree of stimulation of cartilage growth would cause widening, while a stimulation leading to a relative and absolute increase in ossification would produce narrowing, of the epiphysial line. We could show, however, that proliferative processes in the cartilage were predominant in all instances at early stages of administration of the anterior pituitary extract.[2] Therefore, additional local or general factors must be decisive in determining the preponderance of ossification at later stages.

The occurrence of circumscribed osseous plugs in the epiphysial zone, especially in animals which had remained small and had not shown much gain in weight in the beginning of the experiment, gave some indication as to the nature of these factors. These bony enclosures had apparently replaced areas of degenerated cartilage and may therefore be interpreted as secondary processes and not as direct effects of the action of the extract. The degree of ossification in such instances apparently depends, therefore, on the occurrence of more or less extensive degenerations in the cartilage. This interpretation would be additional evidence for the view which we have previously expressed, namely, that ossification in general may be largely dependent on local factors which are active in addition to hormonal influences.[7]

An answer which would apply generally to the question as to why the cartilage is more prone to undergo retrogression in some instances than in others cannot as yet be given. We have been able to demonstrate [4a] that with advancing age the tendency of the cartilage to proliferate under the influence of the anterior pituitary hormone decreases, whereas its tendency to undergo retrogressive changes increases under this condition; but in the experiments which are under discussion the

7. Silberberg, M., and Silberberg, R.: Arch. Path. **28**:340, 1939.

differences in the mode of reaction are obviously due to other factors, because guinea pigs of the same weight and age and treated simultaneously with the same kind and dose of the extract responded to the latter in different ways. The tendency to form bone was, as a rule, greater in animals which had shown less gain in weight. A poor general condition may, perhaps, diminish the power of the cartilage to grow, at the same time intensify the degenerative processes initiated by the hormone, and thus increase bone formation.

In the epiphysial lines of guinea pigs treated over longer periods of time the diffuse calcification of the cartilage which may be observed after injections for from one to three weeks was missing even in animals which had shown little gain in weight. However, the zones of endochondral ossification were narrow in those animals and osseous plugs indicated that degenerative processes had taken place. One might be inclined to consider these bony plugs traversing the epiphysial zone in the direction from the metaphysis as permanent structures preventing in crossbar-like manner further growth of the bones in length. However, this interpretation is not necessarily correct. Some osseous bridges were found in animals which had gained weight steadily up to the time when they were killed, and there is no reason to assume that growth would not have continued if the guinea pigs had been allowed to survive. It is therefore probable that these bony plugs do not prevent further growth in length. Serial sections showed that they may form complete osseous bridges in some places, whereas they are being dissolved by ingrowing bone marrow or proliferating cartilage cells in other places. Resting cartilage cells and even well formed short columns of cartilage cells were seen proximally to the osseous plugs in these cases; the cartilaginous growth originated probably in preserved cartilage cells of the neighboring tissue. If the growth of the cartilage is still further stimulated, the remnants of the osseous plugs may be pushed downward in the direction toward the bone marrow, and may finally be incorported into the mass of newly formed bony trabeculae of the metaphysis. But at the same time increasing sclerosis of the cartilaginous ground substance may lead to the production of new osseous plugs, and thus the competition between these two opposed processes persists. In the end an equilibrium between them may be restored, which guarantees the further growth of the animal in length, and this may counterbalance any shift of this equilibrium which might have occurred during the earlier stages of the experiment.

In those animals which had shown notable gain in weight throughout the duration of the experiments, the second type of reaction to the anterior pituitary extract, namely, a predominance of proliferation over ossification, was seen even at later stages. Wide epiphysial zones per-

sisted for comparatively long periods, and the first symptoms of the formation of bony plugs did not set in earlier than four or more months after the beginning of the experiment.

These differences in the histogenetic mechanism of the reactions of cartilage to the pituitary extract may explain the divergent results recorded by various investigators who studied the effect of anterior pituitary extract on body growth. In chickens Wulzen [8] observed arrest of growth for a period of three months after the beginning of the administration of the anterior pituitary substance. This was probably caused by a long-continued predominance of ossification over growth processes. Schäfer [9] noted in rats, and Robertson [10] in mice, a temporary inhibition of growth followed by normal or even increased growth. These findings agree with our own observations, in which a temporary shift of the balance in favor of ossification was followed by a restoration of the balance with higher rates of both proliferation and ossification. On the other hand, increased growth without initial inhibition as reported by Uhlenhuth [11] in salamanders, Howes [12] and Clements and Howes [13] in axolotl, Goetsch,[14] Evans and Long [15] in rats, Putnam and collaborators,[16] and Teel and Cushing [17] in dogs and Sousa Pereira [18] in rabbits might have been due to a predominance of proliferative processes over ossification for the greater part of the experiment. Finally, the negative findings of Smith [19] and of Larson and co-workers [20] do not exclude the possibility that histologic changes may actually have been present.

From the data of our experiments we cannot conclude that the stimulation of growth exerted by the anterior pituitary extract is maintained indefinitely. To us it seems more likely that the anterior pituitary extract acts in such a manner as to promote the proliferation of the cartilage and its ossification during parts of the physiologic growth

8. Wulzen, R.: Am. J. Physiol. **34**:127, 1914.
9. Schäfer, E. A.: Quart. J. Physiol. **5**:203, 1912.
10. Robertson, T. B.: J. Biol. Chem. (*a*) **24**:385, 1916; (*b*) **24**:397, 1916.
11. Uhlenhuth, E.: Anat. Rec. **23**:43, 1922.
12. Howes, N. H.: J. Exper. Biol. **15**:447, 1938.
13. Clements, D. I., and Howes, N. H.: J. Exper. Biol. **15**:541, 1938.
14. Goetsch, E.: Bull. Johns Hopkins Hosp. **27**:29, 1916.
15. Evans, H. M., and Long, J. A.: (*a*) Anat. Rec. **21**:62, 1921; (*b*) Proc. Nat. Acad. Sc. **8**:38, 1923.
16. Putnam, T. I.; Teel, H. M., and Benedict, E. B.: Am. J. Physiol. (*a*) **84**:157, 1928; (*b*) **85**:40, 1928.
17. Teel, H. M., and Cushing, H.: Endocrinology **14**:157, 1930.
18. Sousa Pereira, L.: Lyon chir. **34**:673, 1937.
19. Smith C. S.: Am. J. Physiol. **65**:277, 1923.
20. Larson, E.; Bergeim, O.; Barber, D. I., and Fisher, N. I.: Endocrinology **13**:63, 1929.

period of the animal and to produce acceleration in the maturation of the skeleton in those animals in which ossification predominates. On the other hand, maturation of the skeleton is delayed if the rate of ossification lags behind that of the intensified new formation of cartilage. The fact that injections of the extract when given over a long period did not produce increased growth beyond the normal adult size, leading to gigantism, may depend on various factors: With increasing time of administration the extract may lose its ability to stimulate tissues, as shown in the case of the ovaries and thyroid by Loeb;[21] this loss of stimulating effect may depend on a gradual change in the reactivity of the epiphysial cartilage under the influence of long-continued administration of the extract.

SUMMARY

Prolonged administration of an acid extract of the anterior lobe of the bovine pituitary exerts a stimulating action on (1) the proliferation and (2) the ossification of cartilage. These effects are similar to those which were noted after administration of this extract for shorter periods of time. The balance between proliferation and ossification may be disturbed in favor of one or the other of these two processes, and this effect may last for some time. If the rate of ossification of cartilage predominates over the rate of new formation of cartilage cells, an acceleration in the maturation of the skeleton may take place; but if the rate of proliferation of cartilage predominates over that of ossification, maturation of the skeleton may be delayed. However, the balance between these two processes can be restored at later stages. The reestablishment of this balance is responsible for the adjustment of skeletal growth. The course of ossification is influenced not only directly by the administration of the anterior pituitary extract but also indirectly by the occurrence and the severity of local retrogressive processes taking place subsequent to the administration of the extract. The histologic differences in cartilage and bone caused by the predominance of proliferation or of ossification of cartilage and the subsequent restoration of a balance between the latter would account for the divergent observations on body growth as reported by different investigators. So-called lines of arrested growth can be observed in the guinea pig, but they do not necessarily coincide with the periods of cessation of weight increase which may have occurred.

Mr. S. J. Hayward made the photomicrographs.

21. (*a*) Loeb, L.: Science **80**:252, 1934. (*b*) Max, P.; Schmeckebier, M. M., and Loeb, L.: Endocrinology **19**:329, 1935.

MALIGNANT ADENOMAS OF THE CHROMOPHOBE CELLS OF THE PITUITARY BODY

ORVILLE T. BAILEY, M.D.
AND
ELLIOTT C. CUTLER, M.D.
BOSTON

There has been much discussion in recent years among clinicians and pathologists in regard to the classification of the more extensive and invasive tumors arising from the chromophobe cells of the pituitary body. Small adenomas taking origin in these cells are not infrequently found in routine postmortem examinations [1]; these usually cannot be correlated with any clinical signs when the patient's history is reviewed. If the tumors of the chromophobe cells of the pituitary body are arranged in a series according to size, they pass from such incidental lesions through tumors causing varying degrees of visual disturbances up to huge neoplasms which destroy the optic chiasm, the normal pituitary tissue and adjoining portions of the skull and cerebral tissue. But does this series represent a single type of tumor? Certainly there is no justification for making subdivisions in a series if size is the only variable component. Or are there among these tumors neoplasms which are sufficiently distinct to warrant separate classification? If there are malignant chromophobe adenomas of the pituitary body, it would be desirable to establish criteria for their identification in the roentgenogram and biopsy specimen, since the accumulation of such data might lead to an appreciation of the special problems in treatment to which these tumors give rise.

The malignant chromophobe cell adenomas have been the subject of relatively few papers, considering the divergence of opinion which has been expressed from time to time by those interested in the subject. As a part of a paper dealing with clinical and pathologic aspects of hypophysial adenomas, Dott, Percival Bailey and Cushing [2] mentioned the malignant adenoma as one of the types of tumors arising from

From the departments of pathology and the departments of surgery of the Harvard Medical School and the Peter Bent Brigham Hospital, Boston, and the Society of Fellows of Harvard University, Cambridge, Mass.

1. Parsons, R., in Medical Papers Dedicated to Henry Asbury Christian, Baltimore, Waverly Press, Inc., 1936, p. 336.

2. Dott, N.; Bailey, P., and Cushing, H.: Brit. J. Surg. **13**:314, 1925.

chromophobe cells and discussed briefly 3 examples, 2 of which were studied in biopsy material only; in the case of the third necropsy was performed. The short statement by Fink [3] contains more valuable information on this subject than many of the longer discussions. Some of the other papers dealing with this question are those of Kux, Redlich and Maxted.[4] Salus [5] described a woman who displayed many of the characteristics of Cushing's syndrome for several years. Eventually she lost ground and died without exacerbation of the symptoms of Cushing's syndrome. At autopsy Salus found a large tumor invading the nasopharynx. The tumor cells did not contain basophilic granules. Was this a basophile adenoma with malignant degeneration? Did the patient have first a basophile adenoma and later an independent malignant chromophobe adenoma which destroyed the first? It is impossible to say. There is little similarity between Salus' interesting case and those dealt with in this paper. The discussion of the possibility of chromophile cell carcinoma falls outside the scope of this paper.

In a study recently published from this clinic [6] the instances of chromophobe adenoma of the pituitary body encountered between Jan. 1, 1928, and Jan. 1, 1936, have been discussed. These amounted in all to 88, 81 of which were verified by biopsy or postmortem examination, and 7 of which were studied only clinically and in roentgenograms. The 81 verified tumors were divided histologically into diffuse and sinusoidal types, a classification suggested by Dott, Percival Bailey and Cushing,[2] which was elaborated, illustrated and correlated with clinical data by us. After this material had been studied, there remained 3 tumors which appeared different from the others. We felt that they should be regarded as a special group to be set apart from the ordinary chromophobe adenomas because of the unusual clinical histories of the patients and the invasive properties and distinctive histologic appearance of the tumors. It may seem to lay undue stress on so small a group to devote a special publication to it. Yet a detailed consideration of the 3 patients and a study of the tumors in the light of general oncologic principles may contribute toward the understanding of a lesion which is one of the most difficult to identify and to treat of any affecting the pituitary body.

CASE 1

Two year history of visual disturbances, impotency, seizures with olfactory prodromes. Extensive destruction of sella turcica shown in roentgenograms. Trans-

3. Fink, E. B.: Tr. Chicago Path. Soc. **12**:359, 1927.

4. (*a*) Kux, E.: Beitr. z. path. Anat. u. z. allg. Path. **87**:59, 1931. (*b*) Redlich, F.: Wien. Arch. f. inn. Med. **30**:111, 1937. (*c*) Maxted, G.: Proc. Roy. Soc. Med. (Sect. Ophth.) **12**:42, 1919.

5. Salus, F.: Ztschr. f. d. ges. Neurol. u. Psychiat. **148**:574, 1933.

6. Schnitker, M.; Cutler, E.; Bailey, O., and Vaughan, W.: Am. J. Roentgenol. **40**:645, 1938.

frontal craniotomy followed by intensive roentgen therapy. Patient alive and at work six and one-half years after operation. Tumor histologically malignant chromophobe adenoma.

Mr. J. T. first came to the clinic Feb. 17, 1933, at the age of 40, because of gradual loss of vision. This had made its first appearance in 1931. In the beginning the difficulty was confined to impairment of vision in the temporal field of the right eye, but there was a steady increase in extent, which was only temporarily improved by successive changes of eyeglasses. In November 1932 the patient found that when he drove his automobile an approaching vehicle would disappear from sight as it came opposite his car. The patient then began carrying out crude visual tests, which showed absence of vision in four fifths of the right visual field and blurred vision in the remainder of that field. He also found that there was no vision in one fifth of the left temporal field of vision. Soon after this he began to experience transient attacks of diplopia lasting fifteen to thirty seconds whenever he turned his head suddenly. The visual disturbances progressed in severity to such an extent that the right eye was almost blind and vision in the left eye was seriously impaired when he came to the hospital.

While at work in August 1932 he had an attack of weakness and dizziness; at the same time he thought he smelled coal gas. He went home at once and fainted shortly after arriving there. Complete unconsciousness lasted for an unknown interval. The patient was not incontinent at the time. On the next day he again stated that he smelled coal gas, but those about him could detect no such odor. The olfactory sensation was followed by a sense of faintness without loss of consciousness. There were four more of these attacks up to December 1932.

In that month, mild headaches in the frontal region made their appearance. These occurred every day and were associated in the patient's mind with eyestrain. At intervals there were also more severe occipital headaches. The patient had no attacks of vomiting; there was no epistaxis or rhinorrhea. He had maintained a weight of 202 pounds (91.5 Kg.). His height was 176 cm. He had 4 normal children. Libido was absent during the two years previous to his admission to the hospital.

Examination.—The patient was alert, well developed and stout, the fat over the abdomen and hips being somewhat more noticeable than elsewhere. The right pupil was larger than the left and reacted sluggishly to light and accommodation. The left showed normal reactions in both tests. There were no abnormalities of movement in the extraocular muscles. The genitalia were well developed, and the body hair followed the distribution normal in men. The tendon reflexes were physiologic with the exception of the right ankle jerk, which was hyperactive. All test odors were recognized. Studies of the visual fields showed almost complete left temporal hemianopia. There was a small area on the nasal side of the right visual field in which vision was preserved, but even the largest test disk could not be perceived by other parts of the right visual field. The corrected acuity of vision was 20/40 in the left eye, 5/200 in the nasal field of the right eye, and 0 elsewhere. Examination of the right optic fundus disclosed a pale optic disk with well defined margins. There was a deep physiologic cup and a visible lamina cribrosa. The left optic fundus was similar except that the optic disk was not so pale. The blood pressure was 135 mm. of mercury systolic and 85 mm. diastolic. The basal metabolic rate was minus 17 per cent. The blood and urine were essentially normal.

Roentgen Observations.—The cranial vault was rather thick and dense. The sella turcica was almost completely destroyed, but the right anterior process

remained. The left anterior clinoid process was not visualized. There was no evidence of a soft tissue mass in the nasopharynx (fig. 1). Roentgenograms of the right hand and wrist showed large bones but no acromegalic changes. The destructive lesion in the sella turcica was regarded as suggestive of a malignant tumor rather than of the usual type of chromophobe adenoma.

Operation.—Feb. 21, 1933, a right frontal bone flap was turned down. The frontal bone was thickened but did not have the appearance of the skull in

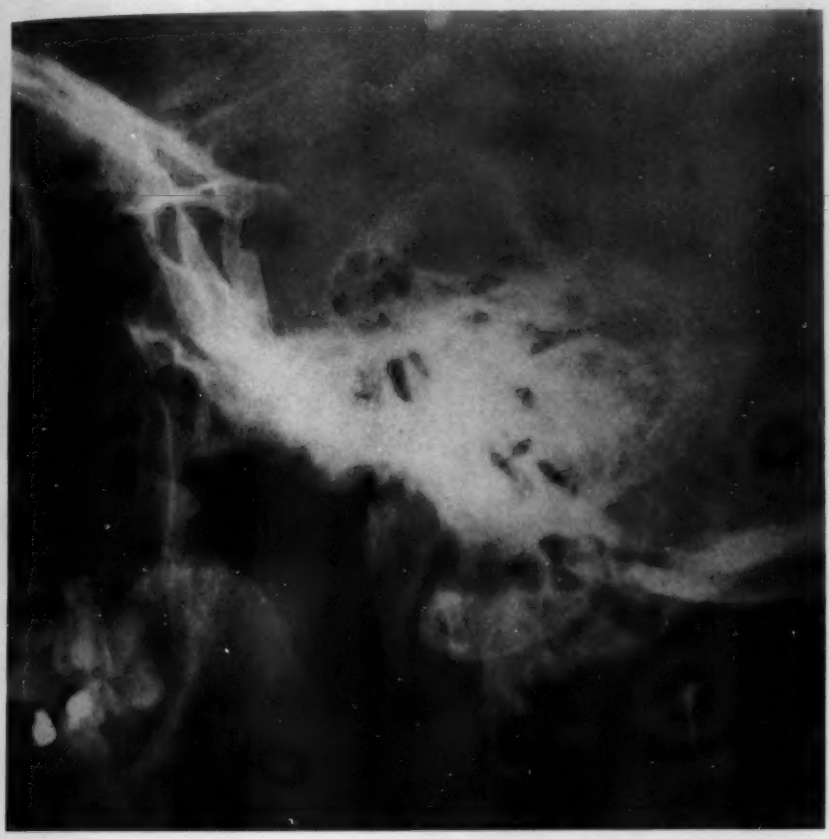


Fig. 1 (case 1).—Roentgenogram showing almost complete erosion of the sella turcica. The amount of bone destruction is greater than that usually seen with chromophobe adenoma of the pituitary.

acromegaly. When the pituitary fossa was brought into view, it was seen to be filled with a meatlike tumor, which flattened the right optic nerve, pushing it upward and outward. Some large fragments were removed for histologic study, and more tumor tissue was evacuated with suction.

Subsequent Course.—Convalescence was rapid and without untoward incident. The visual fields March 8 showed a slight contraction of the defect in the left eye. The patient could distinguish an object the size of the examiner's hand in

the area which was blind before operation. He was discharged March 16. Roentgen therapy was started March 11; between that date and March 15 four treatments, each of 55 per cent of a Holzknecht erythema dose, were given alternately to the right and left temporal portals. The visual fields April 26 showed so much improvement in the left eye that only a small defect remained in the upper temporal quadrant. With the right eye the patient could see the examiner's head on the nasal side of the field but could not recognize the test disks. He felt stronger and the improvement in vision (registered objectively in the visual field charts) enabled him to read better than before operation. Four more roentgen treatments of the same character were given.

June 26, a little more than three months after the patient left the hospital, his visual fields and general condition remained essentially unchanged. He was given another series of four roentgen treatments, the dose being the same as before. After that the patient remained symptom-free except for the defect in vision in the right eye. Since he had no further symptoms, he could not be persuaded to return regularly for observation. In December 1934 he found blood on the pillow when he awoke, which led him to think that he might have had a convulsion during the night. He then returned to find out the cause. The visual fields had remained almost exactly as they were in April 1933. Further roentgen therapy was recommended but refused. One day at the end of March 1935 he suddenly lost consciousness and was told that he had had a convulsion. There was another less severe convulsion a few days later. Because of these, he reentered the hospital on April 6, 1936. Physical and neurologic examinations showed conditions unchanged from those at the time of the previous admission. Roentgenograms of the skull showed that the extent of destruction of the sella turcica had altered little in the three years since the patient first came to the hospital. The left visual field was unchanged from that seen in April 1933. There was perception of light only in the right eye. Roentgen therapy to the amount of 1,170 roentgens was given to each temporal area. A fifth series of roentgen treatments was given in June because of another convulsion and several "fainting attacks." The total dose to each temporal area was 900 roentgens. Repeated visits to June 1937 showed no further change in visual fields and no untoward symptoms. Another series of roentgen treatments was given at that time, the total dose to each temporal region being 1,050 roentgens. Roentgenograms of the skull in June 1938 showed some regeneration of the anterior clinoid processes on the right and slight but definite evidence of healing in all the region of the destroyed bone. At the last examination (February 1939) further improvement in the fields of vision was noted, and no further seizures or headaches had appeared. The patient's weight was 220 pounds (100 Kg.); he remained impotent. He was able to carry on his work as traveling salesman without trouble and had no difficulty with the automobile driving required by it. Roentgenograms of the skull taken at that time showed the pituitary fossa to be about the same size and shape as noted at the first examination, six years previously. The posterior clinoid processes were partly eroded, the right anterior clinoid process was thinned and displaced laterally, while the left was eroded. The left spheno-orbital fissure was larger than the right. The patient received no more roentgen treatment after June 1937.

Biopsy.—The material consisted of 3.5 Gm. of grayish pink tissue in large fragments. It was soft and pulpy. This material was fixed in Zenker's fluid with 5 per cent acetic acid, embedded in paraffin and stained by the following methods: hematoxylin–eosin, eosin–methylene blue, Mallory's phosphotungstic acid–hematein and Foot's modification of the Bielschowsky-Maresch method for reticulum with Van Gieson's stain as a counterstain.

Histologically, the cells of the tumor were massed in broad irregular sheets separated from one another by delicate strands of collagenous stroma. There were many capillaries scattered among the clusters of tumor cells. Most of the strands of stroma were associated with the capillaries, and the collagen seemed rather refractile. The round or oval nuclei of the tumor cells were rich in chromatin. The cytoplasm stained pale and contained very fine granules of a neutral staining reaction. No chromophile cells were encountered. There was moderate variation in cell size and shape from place to place. Mitoses were scattered throughout the tumor in moderate numbers (fig. 2). In one fragment

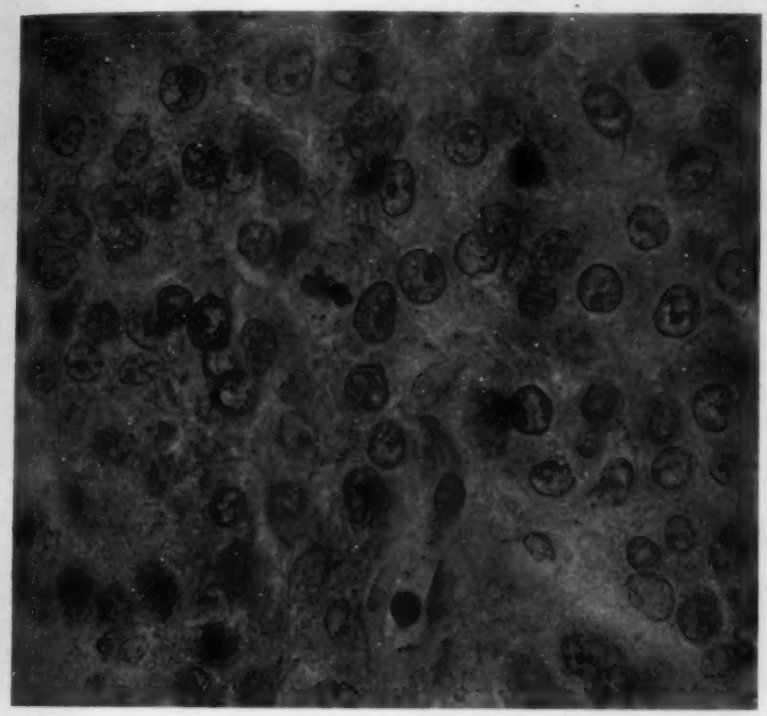


Fig. 2 (case 1).—Photomicrograph of a field in the material removed at operation. The chromophobe cells are closely packed; at the center is a mitosis. Hematoxylin and eosin; × 900.

of the tumor mitoses were especially numerous, two being seen in a single high power field on several occasions. The edge of the tumor was not included in the biopsy material.

CASE 2

Nine months' history of headache, failing vision, convulsions and aphasia. Anosmia, large defects in fields of vision, bulbar paresis. Erosion of sella turcica shown in roentgenograms. Exploration of left temporal lobe with fatality. Autopsy: malignant chromophobe adenoma with extension into left temporal lobe and sphenoid sinus.

Mr. R. P. was admitted to the hospital Jan. 2, 1934, because of headache and difficulty in vision. His difficulties had begun in May 1933, with a steady, burning headache, situated, as he said, "behind the eye." The pain began in the morning and lasted all day; salicylates gave no relief. In the following June he first noticed slight difficulty in vision, but this did not become a major symptom. One month later the patient had an attack of stertorous breathing and could not be made to respond for an hour. The attack was not accompanied by a convulsion, and there were no speech disturbances or other residua after consciousness returned. In October a similar attack was followed by difficulty in speech so severe that he could not be easily understood. He was not able to name objects quickly, though his cerebration seemed otherwise normal. A third attack occurred in November. During this seizure he did not lose consciousness but was unable to speak for about three hours. Speech was then produced in the thick, poorly articulate manner which it had assumed after the second seizure. In December all the patient's teeth were removed. On the next night there was a generalized convulsion, consisting of jerking movements of both arms and legs. The patient was semicomatose during the convulsion and felt drowsy for the next two days. Several similar convulsions occurred during the following week. Nocturia then appeared for the first time. At this period, also, the patient would frequently drop his knife or fork and would be unable to grasp the utensil firmly in either hand when it was returned to him. Walking became troublesome at this period because he could not lift his feet and was compelled to drag them after him. Complete paralysis was not present at any time. Vomiting occurred only once, and then following medication. At the time the weakness of both arms and legs developed, some difficulty in swallowing liquid appeared, and on one occasion regurgitation through the nose followed a fit of coughing. There was at no time evidence of epistaxis or rhinorrhea. The patient had gained 30 pounds (13.5 Kg.) in the previous ten years. He was married fourteen years before; there were 4 normal children. No recent change in libido had been noted.

Examination.—The patient was well developed, somewhat obese, drowsy and in no acute distress. The right pupil was slightly larger than the left; both reacted normally to light and to accommodation. There was weakness in conjugate movements of the eyes to the left. A slow nystagmus was noted when the patient looked either to the right or to the left. There was choking of both optic disks of 1 to 2 diopters, with considerable blurring and hyperemia at the periphery. The visual acuity of the right eye was 20/70; that of the left, 20/100. Charting of the visual fields disclosed nearly complete temporal hemianopia on the right; on the left there was only a small area of vision remaining at the center, since both the temporal and nasal fields were almost completely obliterated. There was normal response to test odors on the right, but complete anosmia was found on the left. There was a suggestion of weakness of the right lower facial musculature. The lower jaw could be only partially opened. The soft palate deviated to the left. Speech was thick and blurred; the patient hesitated for some time before saying the name of any common object. All tendon reflexes were hyperactive but equal on opposite sides. Hoffmann's sign was present on the right but the Babinski sign was not. The hair on the body followed the male type of distribution; the genitalia were normal in development. The blood pressure was 130 mm. of mercury systolic and 80 mm. diastolic. The blood and urine were normal. The spinal fluid was under slightly increased initial pressure. It was yellow and gave a strongly positive Pandy reaction.

Roentgen Observations.—Right stereoscopic and anteroposterior views of the skull showed a vault of average thickness. The pituitary fossa measured 18 mm.

in length and 15 mm. in depth. The left anterior clinoid process was destroyed, and both posterior clinoid processes were rarefied. The left half of the sella turcica was completely eroded (fig. 3). The pineal body occupied a position to the right of its normal situation.

Ventriculograms showed both lateral ventricles displaced to the right of the midline. The left temporal horn was displaced upward and backward. The third ventricle was not definitely outlined, but the air passed freely between the lateral

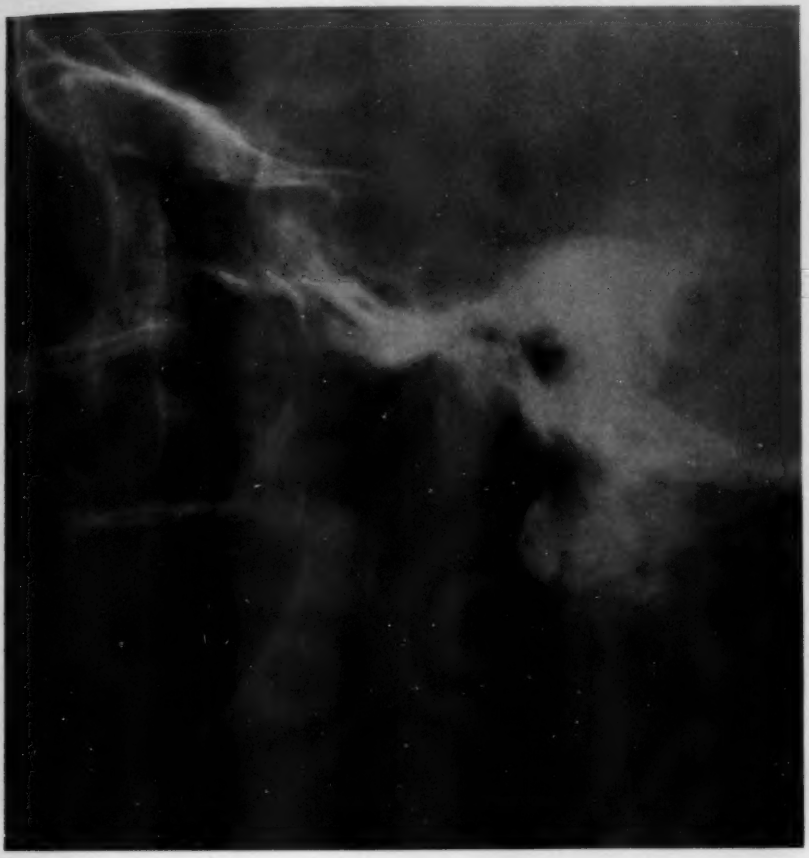


Fig. 3 (case 2).—Roentgenogram showing great enlargement of the sella turcica. The left anterior clinoid process has been destroyed, and the posterior clinoid processes are rarefied.

ventricles. The right ventricle was slightly dilated but was otherwise normal in appearance. This was regarded as indicative of a tumor of the first temporal lobe, probably an extension from a pituitary adenoma.

Operation.—The procedure began with a ventriculographic examination, the results of which have been given. An osteoplastic bone flap was then turned down in the left temporal region, and the area of dura thus exposed was enlarged

by removal of bone as far as the floor of the middle fossa. The dura was under great tension. The convolutions which had been brought into view were flattened and the delimiting sulci almost obliterated. An incision was then made in the temporal cortex at the posterior margin of the field. After about 2 cm. of relatively normal cortical tissue had been passed through, a grayish red tumor of great vascularity was met; it was bleeding actively. To the operator's

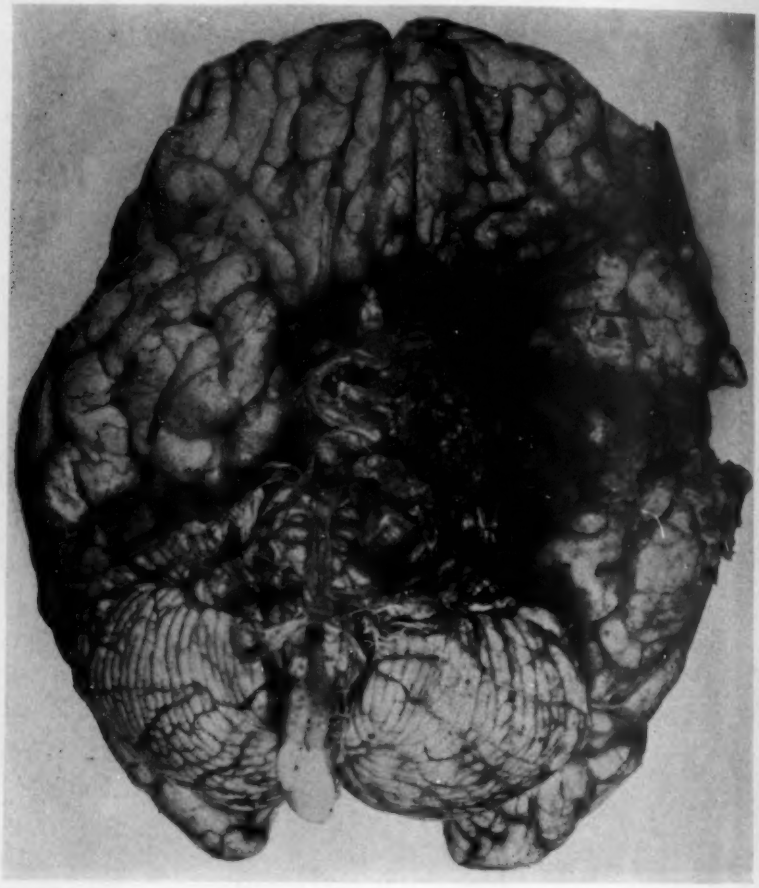


Fig. 4 (case 2).—Photograph of the base of the brain showing the enormous tumor occupying the pituitary region, extending backward over the pons and into the left temporal lobe. The operative defect in the left temporal cortex exposes the deeper portions of the tumor in that location.

examining finger, the tumor seemed well demarcated, lobulated and soft in consistency. Four grams of neoplasm were enucleated. It was then apparent that there was an attachment of the tumor to the base of the brain in the midline near the middle fossa. Further removal of tumor was impossible. The exposed surface of the neoplasm was accordingly seared with the electric cautery, the wound

washed several times with saline solution and the dura reapproximated, leaving a large decompression in the middle fossa. Before the operation the patient was comatose; there was little change at its conclusion.

Postoperative Course.—The patient's condition remained precarious after the operation. It became apparent that the speech center had been affected by the operative procedure, since he could understand and execute simple commands but could not speak. The condition of the patient did not show any definite improvement. On the eighth postoperative day the throat from time to time filled with mucus, but little other change occurred. Then quite suddenly that evening respirations ceased.

Necropsy.—Examination was restricted to the head. When the surface of the brain was exposed by reflection of the dura, the convolutions appeared more flattened in the left hemisphere than in the right. A soft tumor was palpated in the left temporal lobe; this was located beneath the area of maximum convolutional flattening. When the basilar surface of the brain was inspected, attention was at once directed to a reddish gray, extremely soft tumor extending into the left temporal lobe from the region normally occupied by the pituitary body (fig. 4). The mass in the region of the sella turcica measured 5.5 by 5.5 cm.; the medulla was partially encircled by a backward extension, 4 cm. in transverse extent and 2 cm. in anteroposterior diameter. There were many irregular excrescences jutting out from the main tumor. It was an unusually large one of these which produced a tumor bridge to the left temporal lobe, where it formed the mass surgically attacked. The optic chiasma was pushed far to the right of the midline and was partially included in the mass of tumor. The left trigeminal nerve passed directly through the neoplasm and was separated from it with difficulty. The left semilunar ganglion was enlarged to 6 mm. in diameter and was partly surrounded by tumor. Considerable dissection was required to find the rest of the cranial nerves on the left, since all of the first nine either passed through the tumor or had their course altered by it. The cranial nerves on the right side were normal.

The base of the skull was then studied (fig. 5). The cribriform plate of the ethmoid bone was very thin, but there was no extension of tumor to the ethmoid sinus. The frontal sinuses were normal. The sella turcica measured 3 cm. transversely and 2 cm. anteroposteriorly. The right wall was thin and convex in outline. Elevation of the dura in this region uncovered a subdural extension of the tumor, 1 cm. in thickness and 2 cm. in extent, both anteroposteriorly and laterally. There was an irregular anterior extension over the great wing of the sphenoid bone, which resulted in rounding off the left anterior clinoid process and in pushing the right anterior clinoid process farther to the right than normal. Both posterior clinoid processes were also involved. The interior of the sphenoid sinus contained soft, friable, grayish red tumor. There was a small communication between the sinus and the sella turcica to the right of the midline. The petrous portion of the left temporal bone was involved by tumor for 3 cm. adjacent to the sella turcica, but the lateral 4 cm. was free from tumor. The neoplasm extended backward to the occipital bone, which it overlay rather than invaded. The posteriormost tip of the tumor was just anterior to the foramen magnum. The venous sinuses of the dura were free from neoplasm and thrombi. The left internal carotid artery was embedded in the tumor but was not compressed or thrombosed. Careful investigation of the nasal cavity showed it to be free from neoplasm.

After fixation in solution of formaldehyde U. S. P. diluted 1:10 (4 per cent formaldehyde), a coronal section was made, passing approximately through the

center of the brain so as to divide the left semilunar ganglion and the tumor into equal parts and to pass through the third ventricle and basal ganglions (fig. 6). The left lateral ventricle was somewhat compressed, and the left basal ganglions were pushed upward as a whole; the left putamen and globus pallidus were destroyed by the tumor. The left thalamus and internal capsule were slightly distorted by the general upward dislocation of structures by the neoplasm and were infiltrated by the tumor for a short distance inferiorly. The right basal ganglions were normal. A small amount of changed blood covered the ependymal surfaces. The diameter of the third ventricle did not exceed 1 mm. at any point. It was pushed to the right of the midline and curved along the surface of the tumor, but its ependymal surface was free from neoplasm. The only portion of the cerebral cortex affected by the tumor was the left temporal lobe. The operative

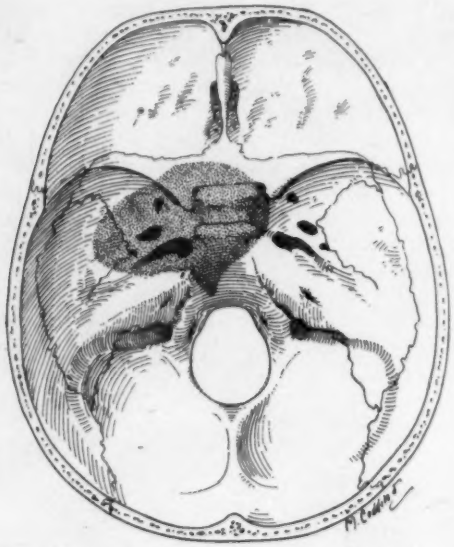


Fig. 5 (case 2).—Diagram to show the extent of bone erosion (stippled). By comparison with figure 4, it may be seen that some of the tumor lay on the surface of intact bone but that the area eroded was large.

incision in this region extended 3 cm. into the cortical tissue. A considerable portion of the subcortical neoplastic mass remained. It was evident that the tumor in the temporal lobe, that in the basal ganglions and that in the sella turcica were directly continuous (fig. 6). The mass in the region of the pituitary body measured in cross section 5 by 4 cm. While most of the tumor surface was dark, irregular and mottled, there were a few white areas, especially along the lower margin, which were firmer than the surrounding tissue. The remainder of the neoplasm was soft even after fixation and showed very extensive degeneration.

Microscopic Examination.—The tumor was examined by the histologic technics listed as used in case 1. On the whole, the type cell of the neoplasm resembled the chromophobe cell of the normal pars anterior. The nuclei were round or slightly ovoid; the nuclear membranes were distinct, and there was a moderate

amount of chromatin. Occasional mitotic figures were found throughout the tumor. The cytoplasm of the type cell took a very pale stain, and on inspection with the highest power microscopic objective it was found to contain many fine granules, which were neutral in staining reaction. The cell walls were not distinct. There was considerable variation in cell size, with a general tendency for the cells to be larger than normal chromophobe cells. No chromophile cells were found in the tumor itself. A very few tumor cells contained small cytoplasmic vacuoles. On the whole the tumor was highly cellular, the cells being arranged in very large groups. The stroma in most areas was composed of small blood vessels and very delicate strands of connective tissue. There was no constant relationship between cells and stroma, and nothing resembling the sinusoids of the normal pars anterior was seen. In some areas, however, there was coarser

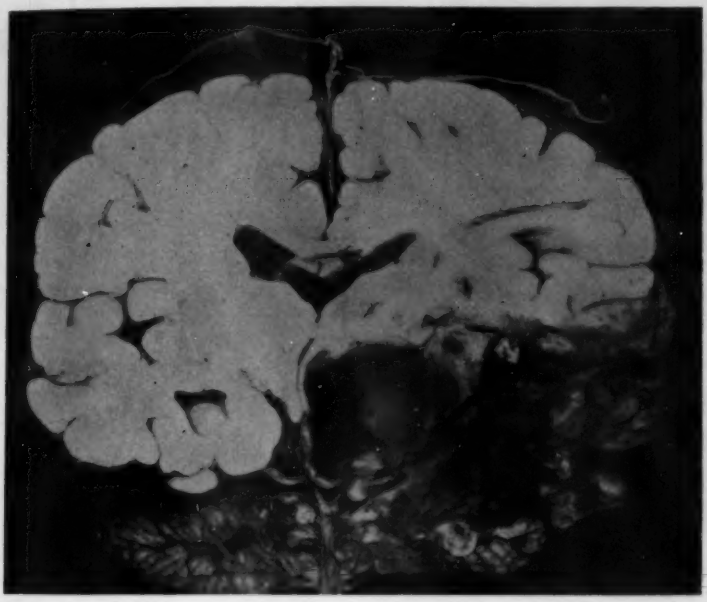


Fig. 6 (case 2).—Coronal section of the brain exposing the tumor in its largest cross section. Note the destruction of most of the left basal ganglions and the distortion of the remainder. This photograph shows that the tumor spread entirely by direct extension.

and more richly collagenous connective tissue. While there were in some places bits of coarse connective tissue from the surrounding dura included within the tumor, the areas with the altered stroma were distinct and the connective tissue newly formed. The new connective tissue appeared to be derived in part, however, from the dura, for when some of the strands were traced out, they could be seen to spread through the tumor from a bundle of dural connective tissue, like the ribs of a fan. In other places, the new stroma originated in the adventitial connective tissue of the larger arteries, an especially good example being seen in connection with the internal carotid artery. The tumor compressed the edge of this vessel and derived some of its stroma from the adventitia, but it did

not infiltrate the inner portions of this layer or affect appreciably the media or intima. The tumor did not grow into the lumens of blood vessels.

The region of the gasserian ganglion and the adjacent portions of the trigeminal nerve were studied. The nerve cells were preserved, but the general architecture of the ganglion was considerably disrupted by the tumor. Masses of neoplastic cells extended into the ganglion and divided groups of nerve cells from one another with little necrosis of nerve tissue. The bundles of nerve fibers in the motor portion were widely separated from one another by tumor, but the individual fibers appeared intact (fig. 7).

Throughout the tumor, but especially in the temporal portions, there were extensive necrosis and hemorrhage. The edge of the tumor where it bordered on basal ganglions and cerebral cortex was sharp. There was little glial reaction in the adjacent cerebral tissue.

An atrophic fragment of the normal pars anterior was included in the material. The sinusoids were compressed and narrow. Well preserved cells of all the normal types were found. However, many of the eosinophils contained small cytoplasmic vacuoles. At the junction of the neoplasm and the fragment of pituitary the tumor cells infiltrated the bit of pars anterior for a short distance.

CASE 3

Ten months' history of nasal obstruction; visual disturbances and headache for six months; diminished sense of smell and amenorrhea. Intranasal growth removed elsewhere, which proved to be extension from pituitary tumor. Complete bitemporal hemianopia; destructive lesion of sella turcica shown in roentgenograms. Transfrontal craniotomy with operative fatality. Autopsy: malignant chromophobe adenoma with invasion of base of skull, sphenoid sinus and right nasal cavity.

Miss Z. H. was a Turkish school teacher, 31 years of age, who came to this hospital for treatment May 16, 1933, after a diagnosis of chromophobe tumor of the pituitary body had been made by her physician in Angora, Turkey.

The first hint of the series of misfortunes which was to befall her was a slight difficulty in breathing which developed in July 1932. Investigation showed that this was due to nasal obstruction. As time went on, this obstruction increased. In March 1933 a surgeon in Angora removed a polyp in an endeavor to improve the condition. On microscopic examination the polyp proved to be a pituitary adenoma of unusual histologic character. Between that time and the time of the patient's arrival at Boston the nasal obstruction increased still further. It was this difficulty in breathing which caused her the most distress throughout her illness.

There was, however, a variety of other symptoms. Beginning in November 1932, she noted a rather severe blurring of vision and a decrease in visual acuity to a degree which prevented her from reading fine print. Coincident with the onset of blurring of vision (i. e., in November 1932) vague, dull headaches occurred from time to time. While the headaches grew more severe as time went on, they were never an important part of the clinical picture. Early in 1933 the patient noticed occasionally a sensation of numbness, tingling and aching in the finger tips of both hands. At about the same time there was diminution in the sense of smell on the right. On careful questioning it appeared that attacks of nausea without vomiting developed as early as 1931 but were not severe enough for her to attach any particular significance to them. In the same year the menses became scanty, and since five months before admission to the hospital there had

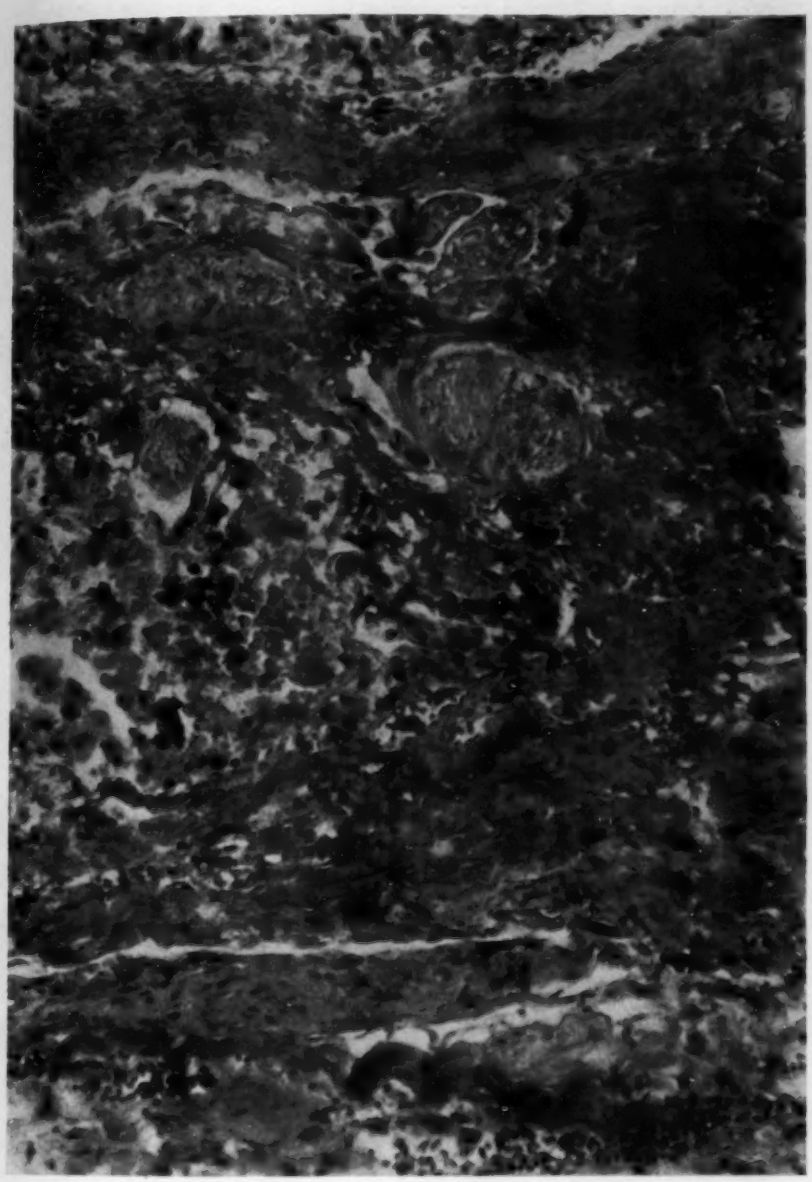


Fig. 7 (case 2).—Photomicrograph of a section through the motor root of the left trigeminal nerve. The individual nerve bundles are intact, though the tumor has separated them from one another.

been no flow. Just before the menses ceased and at intervals after that there were occasions when the patient felt very warm and when blood seemed to rush to the head.

Examination.—The patient was alert, well developed and in no acute distress. The skin was normal in texture; the hair of the body followed the distribution expected in women. The weight of 57 kilograms in comparison with a height of 147 cm. gave a clinical impression of slight adiposity. The extra fat was accumulated over the abdomen and hips for the most part. The pupils reacted to light equally, and there were no abnormalities in the extraocular movements. The visual acuity in the left eye was 20/40 and that in the right eye 20/50. Charts of the fields of vision showed complete bitemporal hemianopia. On examination of the fundus there was slight fibrosis in the lamina cribrosa. Slight tortuosity was noted in the veins of both fundi, but there was no choking of the optic disk. At this time no tumor could be seen in the nasal cavities. A complete physical examination, including neurologic study, showed no further abnormalities.

Roentgen Observations.—The cranial vault was of average thickness. The pituitary fossa was definitely enlarged and appeared expanded. There was considerable destruction of bone, particularly on the left; this also involved the dorsum sellae and the medial end of the left petrous ridge (fig. 8). The sphenoid and petrous ridges were otherwise normal. The sphenoid sinus was cloudy. No definite soft tissue mass could be visualized in the nasopharynx. The impression was that of a rapidly growing, destructive tumor of the pituitary body.

Operation.—The region of the pituitary body was exposed by turning down a right transfrontal bone flap. The greatly enlarged right optic nerve was dislocated upward and laterally by a large tumor. The operator then made a small opening in the tumor by way of the diaphragma sellae; this was followed by an outpouring of neoplasm so soft that it seemed more like a thick fluid than tissue. An endeavor was made to obtain more satisfactory specimens for histologic study with the hypophysial rongeur than could be secured with the hypophysial spoon. The former instrument was accordingly introduced into the center of the tumor and a fragment of tissue gently removed. Immediately the whole field was flooded with bright red blood. The operator at once realized that he had entered the internal carotid artery, which had been involved by the tumor to such an extent that very slight pressure had opened it. The hemorrhage was controlled, at first with gauze, and then with a large piece of muscle. Closure was accomplished successfully. The patient remained in a precarious state and died seven hours after closure had been completed.

Necropsy.—The anatomic diagnoses were: malignant adenoma of the pituitary body, invading the sphenoid sinus and nasal cavities; hyperplasia of the thymus (18 Gm.); miliary adenomas of the adrenals; hirsutism of the lips; focal cicatrization of the pancreas; leiomyoma of the uterus; Ascaris lumbricoides infestation of the terminal portion of the ileum; renal calculi; uric acid deposits in the kidneys.

When the bone flap was elevated, the region of the wound was found free from hemorrhage. The right frontal lobe was then raised from the orbital plate and the operative site brought into view. Near the left side of the tense hemorrhagic tumor the incision made at operation was visible. The reddish soft tissue of the neoplasm pouted through the opening, but there was no accumulation of

blood in this area beyond slight staining of all the tissues at the base of the brain. The upward growth of the tumor had so distorted the optic nerves that both of them at first passed posteriorly and superiorly after leaving the brain substance. They then took a long course forward to each orbit. The left internal carotid artery was displaced from its usual position so as to lie outside the capsule of the tumor, near the operative opening in it. Three millimeters posterior to the incision the wall of the left internal carotid artery was irregular and traumatized. Proximal to the point of injury the artery was distended with a thrombus of recent origin. The right internal carotid artery and cavernous sinus were

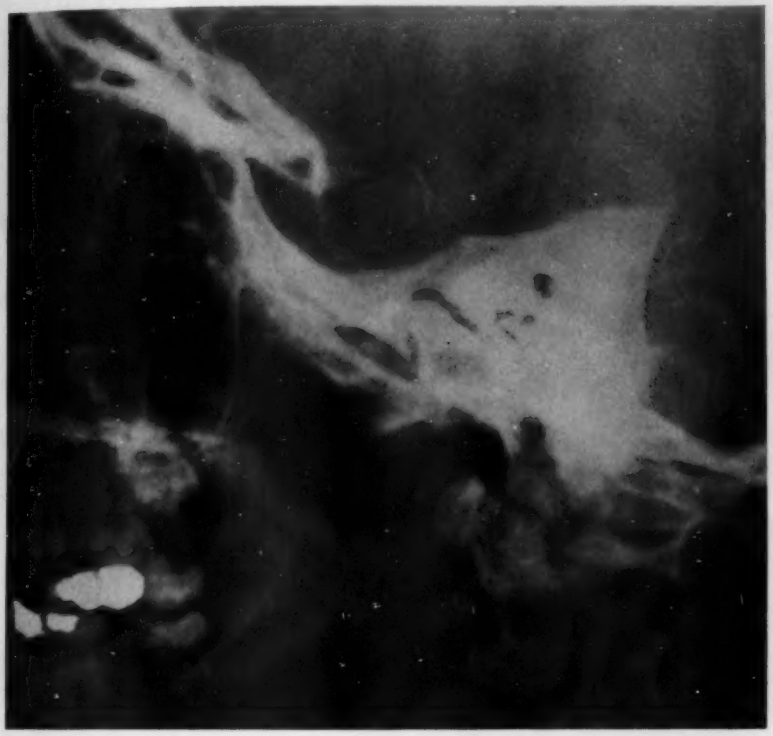


Fig. 8 (case 3).—Roentgenograms showing expansion of the sella turcica with considerable destruction of bone. The cloudiness of the sphenoid sinus proved to be due to a mass of tumor in that location.

included within the tumor, along with the adjacent nerves. There was no evidence of thrombosis or laceration of any of these vessels.

As for the tumor itself, it was situated in the greatly expanded sella turcica and was divided into two approximately equal portions by the sagittal plane of the brain. The main mass of the neoplasm measured 32 mm. in the coronal plane, 18 mm. in lateral extent, and 22 mm. in the greatest anteroposterior plane. The soft, homogeneous, reddish purple tumor appeared entirely encapsulated except in the region of the floor of the sella turcica as will be described. Superiorly the tumor was limited by the compressed floor of the third ventricle; anteriorly,

by the optic chiasm and the anterior commissure; posteriorly, by its distended capsule, the remnants of the thinned dorsum sellae and the posterior commissure (fig. 9).

Fig. 9 (case 3).—Median sagittal section of the brain showing the extent of the tumor and its relations.

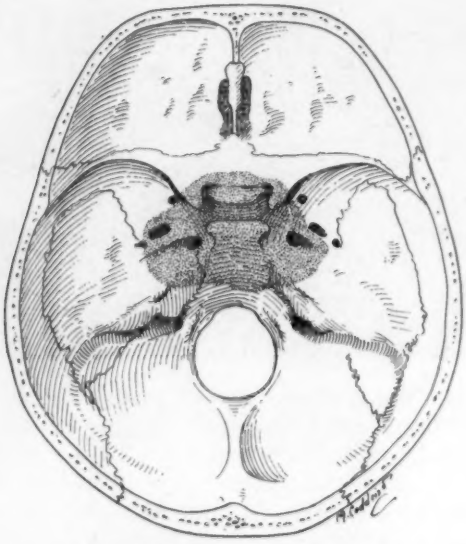


Fig. 10 (case 3).—Diagram of the base of the skull to show the extent of bone erosion (stippled area).

There were widespread erosion of bone and extension of tumor at the base of the skull adjacent to the sella turcica (fig. 10). Both anterior clinoid processes were eroded, and the dorsum sellae was largely destroyed. A perforation was

present in the floor of the sella turcica, in the posterior portion, and the tumor extended through it into the sphenoid sinus. There was a narrow partial septum dividing the sinus into two compartments, each of which was filled with soft tumor tissue in direct continuity with the main mass of neoplasm. The floor of the sinus was defective anteriorly on each side, with the result that a probe could be passed downward into the superior portion of each nasal cavity. Inspection from below showed that there were two defects, each 2 mm. in diameter, on the left side of the nasal septum above the superior turbinate. No tumor tissue was found within the left nasal cavity. In the right nasal cavity a nodule of

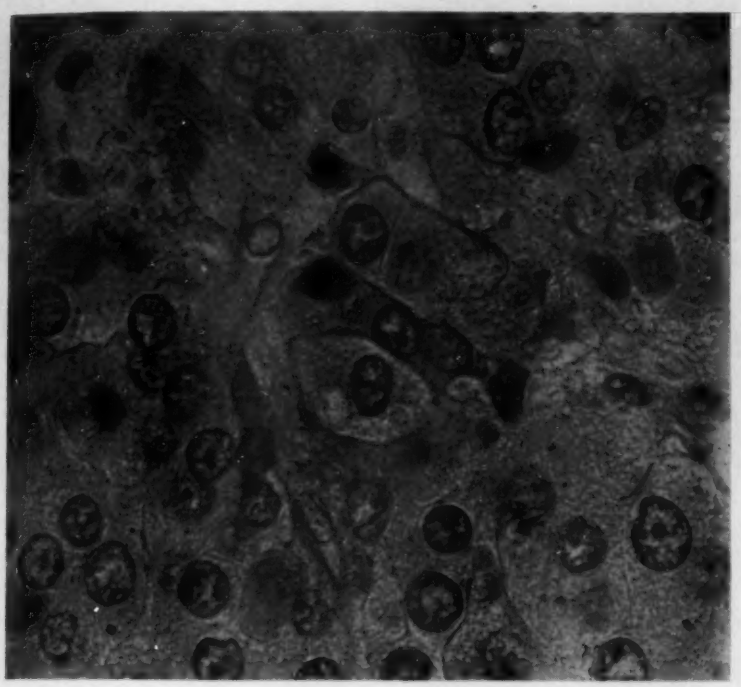


Fig. 11 (case 3).—Photomicrograph to show the morphologic variation in the cells. Note the cell with the vacuoles at the center. This appears to be an earlier stage of the vacuolation shown in figure 12. There is a mitosis at the left. Hematoxylin and eosin; × 900.

neoplasm extended for a distance of 8 mm. All the tumor tissue was directly continuous, and no metastases were found in the skull or elsewhere in the body.

After fixation in solution of formaldehyde U. S. P., the brain was cut in the midline anteroposteriorly (fig. 9). The tumor was soft and varied in color from reddish purple to pale gray. Along the anterior aspect of the neoplasm there was a thin gray line, which proved to be a portion of normal pituitary tissue. The floor of the third ventricle was thinned from compression by the tumor, and there were several defects in it in the region of the intermediate mass and others near the posterior commissure. The third ventricle itself was not dilated.

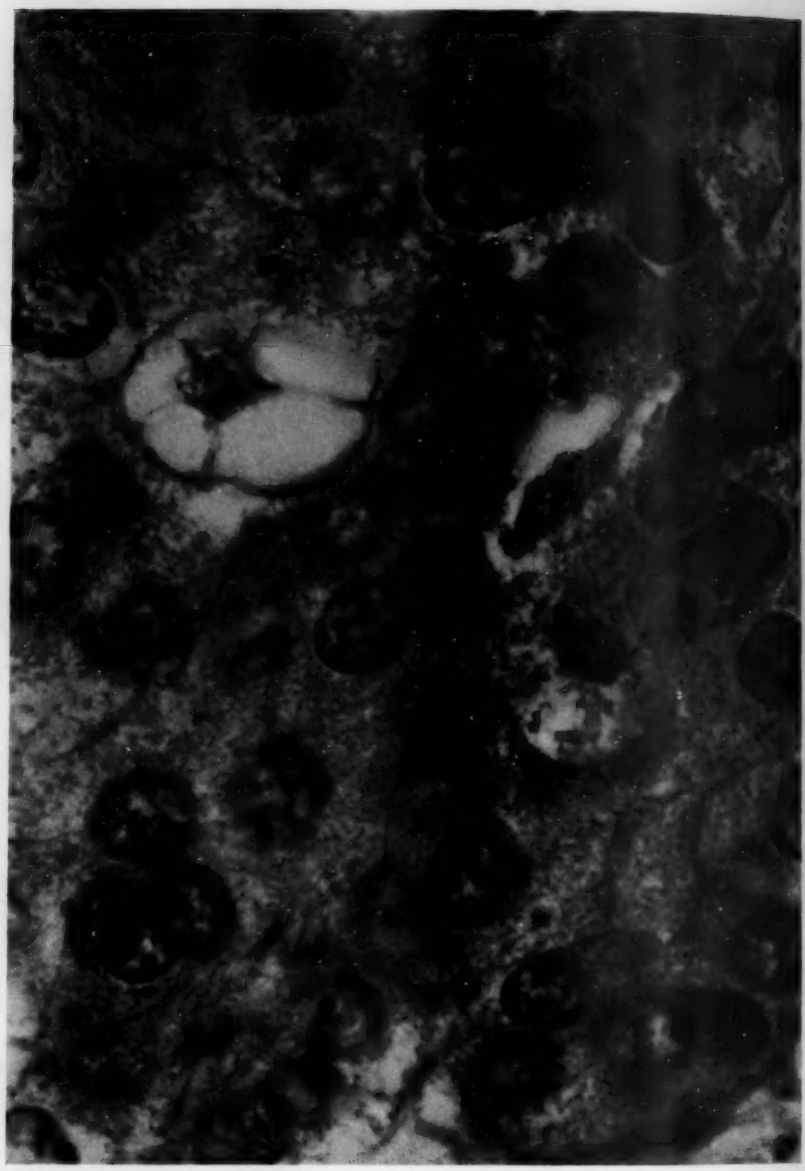


Fig. 12 (case 3).—Photomicrograph to show details of cell structure. A vacuolated cell of the type discussed in the text is shown just above the center of the photograph. There is a mitosis at the lower right. Hematoxylin and eosin; × 1,700.

The pineal gland was situated 10 mm. posterior to its usual site but otherwise was not altered. The aqueduct of Sylvius measured 1 to 2 mm. in diameter. The fourth ventricle was normal.

Microscopic Examination.—The material available for histologic study had been obtained (1) at the operation on the nose in Turkey, (2) at the operation in this hospital and (3) at necropsy. Sections from all three lots of material were prepared and stained by the methods listed in the report of case 1. The sections of material obtained at the second operation and of that obtained at

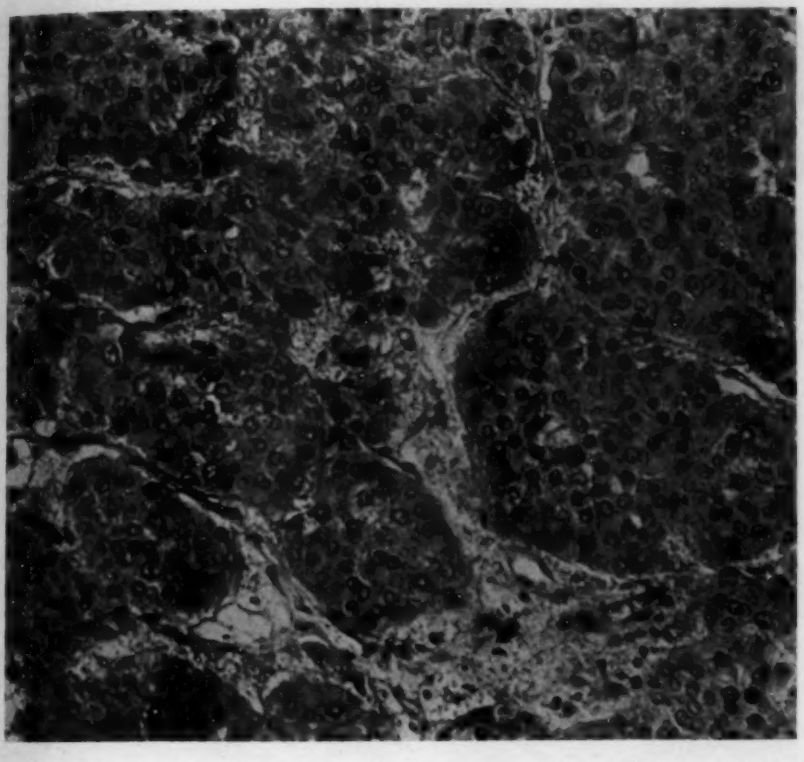


Fig. 13 (case 3).—Photomicrograph of the tumor showing the formation of clusters of cells separated by a coarse collagenous connective tissue, quite different from that of the normal pars anterior. Hematoxylin and eosin; × 300.

necropsy presented no essential differences and are described together. The sections of the material from the nose showed certain variations, which are discussed separately.

The nucleus of the type cell was round and contained a moderate amount of chromatin. The cytoplasm took a light stain, which appeared uniform until it was studied with the highest power microscopic objective; then fine granules, neutral in staining reaction, could be resolved. The cell wall was more distinct than that of the usual chromophobe cell. The cytoplasm of occasional cells contained clear vacuoles (fig. 11). These usually occurred singly, though in some

cells the cytoplasm was filled with vacuoles (fig. 12). The cells with vacuolated cytoplasm were scattered rather uniformly throughout the tumor and were not associated with areas of necrosis. Even when vacuoles were not present, there was considerable variation in cell size and shape. Mitotic figures were present in moderate numbers throughout (figs. 11 and 12). No chromophilic cells were seen.

Although the tumor was richly cellular, there was in many areas a tendency toward arrangement of tumor cells in small masses (figs. 13 and 14). Among the cell masses were strands of connective tissue, loose in texture and different in

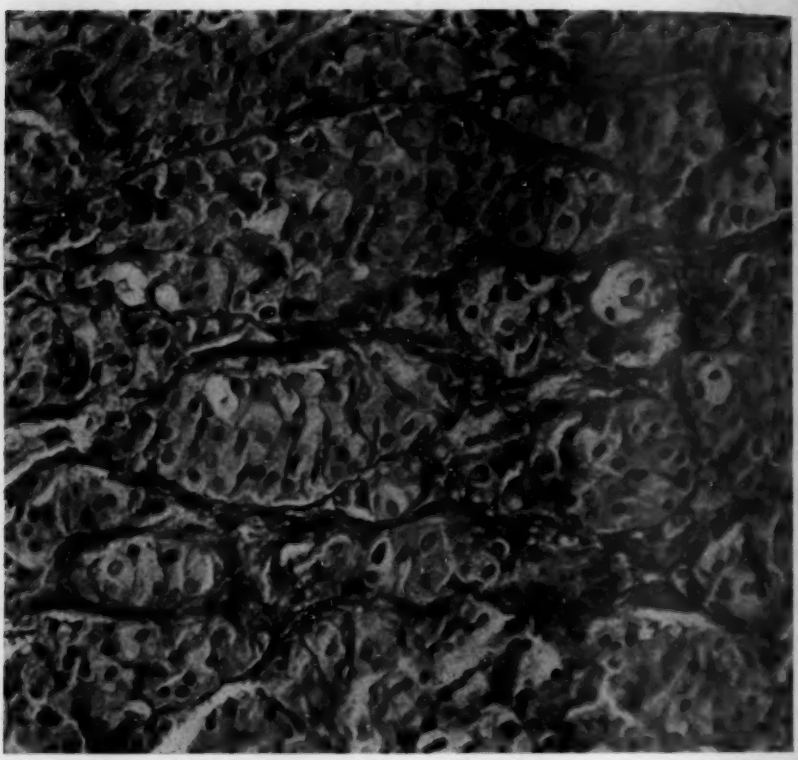


Fig. 14 (case 3).—Photomicrograph of an area in which there were numerous vacuolated cells. (See figs. 11, 12 and 15.) Note the coarse connective tissue separating the closely packed groups of cells from one another. Hematoxylin and eosin; × 300.

character from that of the normal pituitary body. Only seldom were the cell groups penetrated even by the smallest collagen fibers (reticulum). This stroma did not resemble dural connective tissue and was definitely not old connective tissue included by the extending tumor. Moderate numbers of small capillaries were scattered through the tumor, but they also seldom penetrated into the cell masses. The dural margin was rather uniform along the tumor edge; no small clusters of cells extended into it. One large vessel in this region showed marked

endothelial proliferation, but elsewhere the walls of vessels were normal. There was no intravascular growth of neoplastic tissue. Extensive necrosis and some hemorrhage were found in the tumor, part of the latter being postoperative.

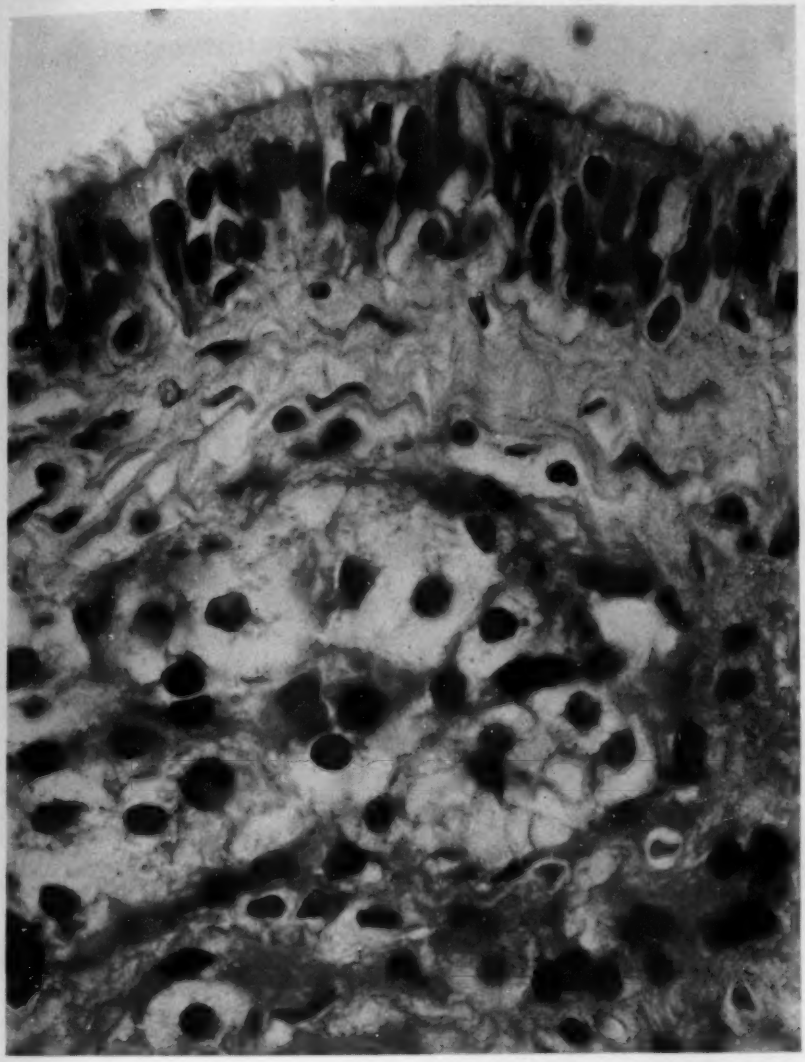


Fig. 15 (case 3).—The edge of the intranasal projection of the tumor is shown. The nasal mucosa is intact. Just beneath it are closely packed tumor cells, most of them vacuolated. Hematoxylin and eosin; × 900.

The material removed from the nose in Turkey was somewhat different in appearance. The superficial surface was covered by ciliated epithelium, which was intact in most places (fig. 15) but showed here and there small ulcerated

areas. In the latter areas the edge of the tumor extended to the surface, but in other places it was separated from the normal mucous membrane by a narrow band of collagenous connective tissue. The tumor cells were grouped in discrete masses, separated from one another by strands of connective tissue. This stroma

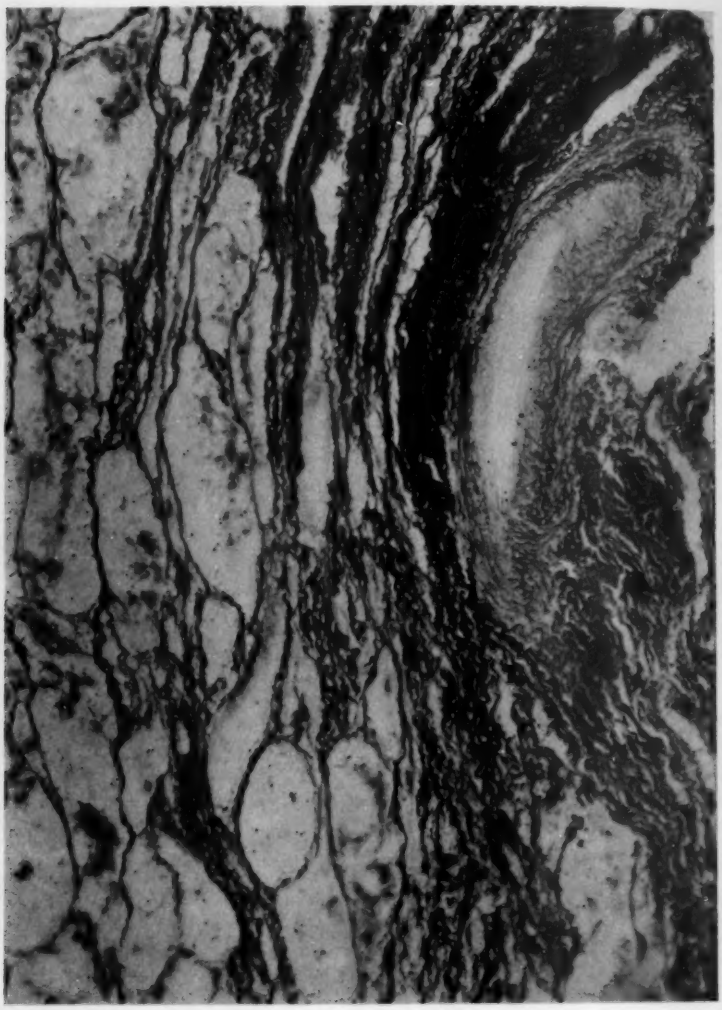


Fig. 16 (case 3).—Photomicrograph to show the behavior of the stroma at the edge of the tumor. Foot's method for reticulum; × 300.

was derived from the connective tissue of the nasopharynx. Figure 16 gives an indication of the basis for this statement, though the conclusion was reached only after study of many sections stained in various ways. The original connective tissue consisted of bundles of closely packed parallel wavy fibers, which stained

dark red with van Gieson's stain but were not impregnated by Foot's method for reticulum. The stroma of the tumor was more delicate in texture and was deeply impregnated by the silver method. In places where continuity between the two could be seen, the new connective tissue grew out of the old irregularly and extended in all directions from the parent tissue. The individual cells in the nasal extension were much like those of the center of the tumor, though the percentage of vacuolated cells was higher in the material from the nose. Mitoses were frequent in this portion. Unfortunately, none of the material was fixed suitably for glycogen stains, and the specimen was too old when this study was undertaken to permit the use of reliable fat stains. Therefore it was impossible to determine the content of the vacuolated cells by selective staining methods.

COMMENT

The foregoing pages contain descriptions of three unusual tumors arising from the chromophobe cells of the pituitary body. The patients would be exceptional among those suffering from pituitary tumors if only for the marked departure of their clinical course from that of patients with the syndromes usually caused by such lesions. The common characteristics which tend to set their tumors apart from the usual variety of chromophobe adenoma are their short history and the evidence of rapid extension into both the bone and the neighboring brain substance. A more detailed consideration of the gross and microscopic appearances of the three tumors and of certain tumors described in the literature points to the conclusion that they represent a small but distinct group—the malignant chromophobe adenomas of the pituitary body. They possess many of the criteria used by general pathologists in the diagnosis of malignancy in a tumor of any organ. Yet they do not metastasize if, indeed, the case of Dott, Percival Bailey, and Cushing [2] is excepted. This case is discussed later. What is a tumor to be called which is locally malignant but which rarely, if ever, metastasizes? We have chosen to use the term "malignant adenoma" rather than "adenocarcinoma," the only two apparent alternatives unless a term be coined. The use of the term "malignant adenoma" brings the classification of the tumors in line with that used by Cutler and Gross [7] for tumors of nerve sheath origin. They have called the entirely encapsulated lesion neurofibroma, the locally invasive tumor malignant neurofibroma and that producing widespread metastases neurofibrosarcoma. The malignant adenoma of the pituitary body and the malignant neurofibroma each represent neoplasms which are locally invasive but which do not produce distant metastases.

We return to the histologic characteristics of this group of tumors. In the type cell the cytoplasm is abundant, but its limits are often poorly defined; it is filled with minute granules, which stain very lightly and are not strongly acidophilic or basophilic. The nucleus is round or

7. Cutler, E., and Gross, R.: Arch. Surg. **33**:733, 1936.

oval, usually with one prominent nucleolus. These are all characteristics of the familiar chromophobe cells of the normal pars anterior and of the usual type of chromophobe adenoma. In the 3 cases discussed here, however, the individual cells diverged in certain respects from this pattern. Many cells were larger than normal chromophobe cells, while some were considerably smaller than the normal ones. On the whole, the tumor cells were closely packed in broad sheets and irregular masses. In case 3 there were vacuoles in the cytoplasm of many of the tumor cells in all parts of the neoplasm but especially in those in the intranasal portion (figs. 11, 12, 14 and 15). Similar vacuoles were found in a few tumor cells in case 2 but not in case 1 or in the 81 cases of benign chromophobe adenoma of the pituitary body reported previously.[6] The vacuoles varied in size from small droplets (as in the cell near the center of fig. 11) to a size so large that several occupied almost the entire cytoplasm (fig. 12). Vacuoles were not found within the nuclei. Studies have failed to demonstrate the exact nature of these cytoplasmic droplets. Cells containing the vacuoles showed no evidence of fragmentation or nuclear disintegration, which makes it unlikely that the vacuoles were the result of cell degeneration. In all tumors of this series mitoses were seen in moderate numbers. In some chromophobe adenomas of the usual type, a few mitoses can be found after search. However, the mitoses in the three tumors to which this study is devoted were scattered through all parts of the tumors in greater numbers. There were, however, a few foci in which mitoses were especially numerous, the only characteristic which seemed to display significant variations in different parts of the tumors. Cell type, arrangement and stromal relationships retained that uniformity which has been illustrated in a previous paper.[8] In summary, then, the cells in all three tumors were derived from the chromophobe elements of the pars anterior but differed from them and from the cells of the usual chromophobe adenoma in variation in size, with a tendency to be larger than normal, in irregularity of arrangement and in the frequency of mitotic figures. These are cellular characteristics commonly regarded as suggesting rapid growth in any tumor.

The character of the stroma in the three tumors under consideration seemed even more definitely to indicate that they should be set apart from the usual chromophobe adenomas. The chromophobe cell is an epithelial cell. As such, it must be in close relation to a supporting and nutritive stroma. This, it should be borne in mind, is just as essential for the growth of neoplasms originating from epithelial cells as it is for normal development. Now general pathologists have long recognized that the stroma called forth in a malignant tumor of any organ differs from the normal stroma of that organ. Any structural peculiarity

8. Schnitker, Cutler, Bailey and Vaughan,[6] figures 1 to 4.

or arrangement of fibers peculiar to the stroma of the organ in which the tumor takes origin is suppressed or disappears entirely. As a result of these changes, the stromas of carcinomas arising in such diverse structures as the endometrium and the epithelium of the breast tend more nearly to resemble each other than to simulate the appearance of the original stromas. In each of the 3 tumors under discussion the stroma was more irregularly distributed, coarser and more refractile than that of the normal pars anterior or of the usual chromophobe adenoma. Figures 13 and 14 show the dense connective tissue stroma and the tendency for it to separate the tumor cell masses from one another. Particular care was taken to be certain that this change in character of the stroma was present in various parts of the neoplasm and to exclude the possibility that the coarse, refractile fibers could have been a part of the connective tissue of the dura included in the expanding tumor. At the edge of the neoplasm in cases 2 and 3 there was definite evidence that the stroma was derived from the connective tissue of the organs infiltrated by the tumor and that it was not carried forward by the tumor. The power to stimulate the growth of connective tissue in a region distant from the site of origin of a tumor is an indication of malignancy. The edge of the neoplasm in case 1 could not be studied because only biopsy material was available.

There is little of special note in the arrangement and appearance of the blood vessels in malignant chromophobe adenomas. They are very vascular tumors, but so are the benign chromophobe adenomas. There is no special relationship of cells to blood vessels as in the sinusoidal type of benign adenoma.[6] In this material there was no instance of intravascular growth of tumor. Though the tumors were highly vascular, there were numerous large areas of necrosis. The necrotic areas showed complete destruction of tumor tissue, often with evidence of old hemorrhage as well as that incident to the surgical procedure.

It may be seen from this discussion that the segregation of malignant from nonmalignant chromophobe adenomas has been made independent of the size of the neoplasm. On the other hand, it is to be expected that the more rapid and infiltrative the manner of growth, the larger the tumor will be by the time the patient presents himself at the clinic. This is borne out in the reasons given by the patients for consulting a physician. In case 1 the presenting symptom was difficulty in vision, just as it is in most cases of benign chromophobe adenoma. In case 2 the patient was first troubled by headache and some difficulty in vision, but neither were severe enough to make him come to the clinic; he waited until he had an uncinate seizure. In the third case the first symptom which the highly intelligent patient regarded as significant was nasal obstruction. Thus attention was first directed in 2 of 3 cases toward a mass of tumor which was the result of extension of the growth

beyond the region of the sella turcica—in the first instance, into the temporal lobe, and in the second, into the nasopharynx.

The infiltrative character of malignant chromophobe adenoma is well indicated by the extent of destruction of the skull. Erosion of the sella turcica is common with all pituitary tumors except the smallest, and many of the neoplasms arising from chromophobe cells which we classify as benign are associated with considerable involvement of neighboring portions of the skull. In each of the 3 patients of this group, however, there was wide destruction of the skull bones, which seemed to offer little resistance to the tumor (figs. 5 and 10). Such free invasion allows the tumor to spread out more readily than a tumor of the benign type. Accordingly, the symptoms referable to involvement of the sellar region per se are apt to develop later and to be less striking when the lesion is malignant than when it is benign.

The roentgenograms of patients with malignant chromophobe adenoma sometimes show clouding of the sphenoid sinus, indicative of extension of the tumor there, as in case 3. This and the destructive character of the sellar lesion are valuable aids in the preoperative differentiation of the malignant from the benign adenoma of chromophobe type.

It is evident that a tumor of the pituitary body which so widely invades the sphenoid bone and neighboring parts of the skull must come into direct relation with important vessels and nerves. The optic tracts are usually pushed upward by an expanding pituitary tumor of any type. This may be somewhat less noticeable in some patients with malignant chromophobe adenoma because of the freer extension of such a tumor into the skull bones and surrounding structures (for example, case 2). The tumor also comes in close contact with the internal carotid artery and the cavernous sinus. The benign chromophobe adenoma tends usually, but not always,[2] to push aside these important structures, while the malignant one may be expected more frequently to incorporate these vessels within the advancing neoplasm. This was well shown in the postmortem material in case 2, in which the tumor cells extended into the adventitia of the internal carotid artery but did not involve its media. In case 3 this sort of extension led to a situation which precipitated an operative fatality. While there is no instance in this series, further growth in this location might well result in occlusion of the internal carotid artery and consequent encephalomalacia, especially if the circle of Willis was compressed at the same time. Cranial nerves of the region about the sella turcica other than the second may be involved by the tumor in such a way that they become ensheathed by the neoplasm. The continuity of individual nerve fibers is usually preserved (fig. 7), but further involvement of this kind serves to explain the cranial palsies

which occasionally accompany a tumor of this type. The semilunar ganglion was invaded by the tumor in case 2. Its architecture was partly disrupted, but the nerve elements showed little, if any, necrosis.

When the malignant adenoma grows upward, it has free access to the temporal lobe. Here the tumor may form a secondary mass larger than the growth in the region of the sella turcica. It is important to bear in mind that the two masses are connected by a bridge of neoplasm and that the involvement of the temporal lobe is by extension and not by metastasis. The tumor bridge in case 2 is illustrated in figure 6. The involvement of the temporal lobe brings on a change in symptoms. As pointed out by Spiller,[9] careful neurologic study of patients with this type of lesion enables one to predict the extent and relationships of the tumor with great accuracy. He pointed out that the well known uncinate syndrome is the result of a lesion causing irritation and, furthermore, that destruction of the uncinate region leads to homolateral anosmia and agusia, progressive paralysis of the opposite side of the body and other symptoms, depending on the extent of the lesion. The importance of recognizing the possibility of extension into the temporal lobe is emphasized by the fact that the symptoms indicative of involvement of the uncinate region may be more striking than those of the original tumor. In such instances as case 2, the discrepancy between the symptoms caused by the primary growth and those caused by the temporal extension has led to operation directed toward the extension and not toward the primary growth.

This is not the place for a discussion of the differential diagnosis of chromophobe adenoma of the pituitary body as a whole. Reference is made to the papers of Biggart and Dott,[10] which contain a full discussion of this topic. There are a few special problems in differential diagnosis which must be discussed in this paper because they attain particular prominence in chromophobe adenoma of the malignant type. Methods for differentiating the benign from the malignant adenoma have been suggested in various places but may be summarized here. Ocular symptoms are frequently earlier and more severe in the presence of a benign than in that of a malignant adenoma because of the tendency of the latter to spread more freely outside the confines of the sella turcica. Attention is frequently attracted first to a malignant chromophobe adenoma because of symptoms of its more distant extension, as nasal obstruction or an uncinate seizure. Further evidence of the presence of a malignant chromophobe adenoma may be secured from the roentgenogram, which shows destruction of bone with extension to the temporal and even the occipital bones rather than the more localized

9. Spiller, W.: Arch. Neurol. & Psychiat. **16**:73, 1926.

10. Biggart, J., and Dott, N.: Brit. M. J. **2**:1153 and 1206, 1936.

enlargement of the sella turcica with displacement of adjoining structures seen with benign chromophobe adenoma. A soft tissue mass in the sphenoid sinus or in the nasopharynx is, of course, indicative of the malignant type of adenoma. A ventriculogram may demonstrate the presence of a temporal tumor, which when combined with evidence of enlargement and erosion of the sella turcica is presumptive evidence of a malignant pituitary adenoma with extension to the temporal lobe. While the final decision must rest on histologic study, it should be possible to decide with a fair degree of accuracy whether a chromophobe adenoma of the pituitary body is benign or malignant before operation is undertaken.

There is one type of tumor involving the sellar region which is much more apt to be confused with the malignant than with the benign chromophobe adenoma, the chordoma. Both the malignant chromophobe adenoma and the chordoma cause extensive destruction of the sphenoid and occipital bones, and each may extend into the nasopharynx. The manner of involvement of the cranial nerves may be much the same in both cases. However, the chordoma does not usually give rise to hypopituitarism.[11]

Carcinoma arising in the lining of the sphenoid sinus and carcinoma having its origin in the epithelium of the nasopharynx must also be differentiated from malignant chromophobe adenoma. From either of these sites carcinoma in unusual cases may grow into the sella turcica and destroy the pituitary body wholly or in part. The tissue in the nasopharynx or in the sphenoid sinus may resemble grossly extensions of a tumor arising in the pituitary body. The resemblance between the carcinoma of the nasopharynx or the carcinoma of the sinus epithelium and the malignant pituitary adenoma is not great as a rule. Confusion arises when the examiner is seeking the origin of a large tumor involving the sella turcica, the sphenoid sinus and the nasopharynx. We have encountered 2 instances in which a tumor involving these three structures arose from epithelium of the respiratory tract. One of the patients was a man of 52 who suffered from loss of taste and smell, nosebleed and protrusion of the left eyeball. On examination he was found to have bitemporal hemianopia and choked disk. Roentgenograms revealed erosion of the left clinoid process and of much of the rest of the sphenoid bone and the left orbital plate. The tumor was demonstrated by biopsy to be a carcinoma arising in epithelium of the respiratory tract. The tumor unexpectedly showed a very satisfactory response to intensive roentgen therapy, and the patient is in good condition four years after the biopsy was made. The second patient (the following data are quoted from the records of the Albany Hospital by permis-

11. Hass, G.: Arch. Neurol. & Psychiat. **32**:300, 1934.

sion of Dr. A. W. Wright) was a youth of 17 who presented progressive emaciation and enlargement of the liver. At necropsy there were many discrete nodules of carcinoma in the liver, obviously metastatic. The only other tumor was a large mass involving the sphenoid bone and the pituitary body; the latter had been destroyed by it. The impression at the necropsy table was that the neoplasm arose in the pituitary body and metastasized to the liver. On microscopic examination, however, the tumor cells were found to be undifferentiated but bearing no resemblance to chromophobe cells. A careful search finally showed among them certain cells with cilia, which established the relationship to epithelium of the respiratory tract. We believe that the patient mentioned by Dott, Percival Bailey, and Cushing [2] as having a malignant chromophobe adenoma of the pituitary body which metastasized to the liver probably had a tumor similar to the one in the second of these cases. In their paper they specifically mentioned alveolar arrangements of cells resembling those of the thyroid gland. We are hesitant in accepting as malignant chromophobe adenoma tumors in which there are large distinct alveolar groupings of tumor cells. In such instances careful study of material which has been well fixed and stained will often reveal cuticular borders or cilia, either of which make it certain that the tumor does not arise from chromophobe cells. Squamous or transitional cell carcinoma may also arise in this region, either from the nasopharyngeal epithelium or from remnants of Rathke's pouch.

The differentiation of carcinoma originating in the sphenoid sinus or in the nasopharynx from malignant chromophobe adenoma is important in determining whether the pituitary tumor ever gives rise to distant metastases. Carcinoma of the types here enumerated often metastasizes to the cervical lymph nodes, lungs, liver and other organs. On the other hand, in neither of our cases of malignant chromophobe adenoma studied at autopsy were there distant metastases even though the primary tumor was large. A survey of the literature relating to malignant pituitary adenoma indicates that the only instance of such a tumor with metastases reported was that described by Dott, Percival Bailey, and Cushing,[2] which, we feel, should be excluded from the group. Malignant chromophobe adenoma may invade a large area directly, but distant metastases have yet to be demonstrated. We have not seen implantations within the cerebrospinal axis.

The treatment of a malignant chromophobe adenoma presents a number of difficult problems. Because of the situation of the neoplasm, a complete surgical extirpation is impossible. Partial removal of the growth, however, can be performed by the same technic as that used in dealing with a benign chromophobe adenoma. There are several reasons why partial removal of a malignant adenoma may be less satisfactory than that of a benign chromophobe adenoma. The wide extension of the

malignant adenoma through the skull provides a partial decompression of the sella turcica; hence, the improvement following sellar decompression is greater with the benign than with the malignant adenoma. Since the malignant adenoma grows more rapidly than the benign, any improvement resulting from partial removal might be expected to be more temporary. Furthermore, the tendency of the malignant adenoma to surround the large blood vessels about the sella turcica, such as the cavernous sinuses and the internal carotid arteries, makes serious damage to them a distinct possibility. Surgical exposure of the tumor may be necessary for diagnosis and should be done if careful neurologic study and roentgenograms still leave the nature of the lesion in doubt. It is sometimes possible to remove a portion of an intranasal extension of the growth for histologic study, as was done in case 3.

Roentgen therapy is an agent of definite value in the treatment of these tumors. Our technic and our results with benign chromophobe adenoma have been summarized elsewhere.[6] The material at our disposal does not permit any final statement as to the ultimate value of roentgen therapy in dealing with malignant chromophobe adenoma. However, the only patient in whom we have given it a thorough trial (case 1) has done so well that we are encouraged to try it in more cases of this type. When the diagnosis can be made with certainty from clinical and roentgenologic studies, we suggest irradiation without preliminary surgical procedures, reasoning by analogy from our results with benign chromophobe adenoma.[6] The problem of treatment is even more difficult when there is an extension of the tumor in the temporal lobe. Surgical attack on the mass in the temporal lobe is a possible palliative procedure (as in case 2). Roentgen therapy has been successful in case 1 so far in controlling symptoms which point to involvement of the temporal lobe.

A word of caution is necessary in regard to the treatment of an intranasal extension of a growth of this type. When radium is applied to the mass through the nose, the result is often a fatality, because of the uncontrolled necrosis and subsequent infection.

Malignant adenoma of the chromophobe cells of the pituitary body thus presents problems in treatment which are different in certain respects from those of benign chromophobe adenoma. It is, therefore, important to separate instances of the malignant type of adenoma into a distinct group. Only in this way can data be accumulated so as to lead to improvement in the diagnosis and treatment of this type.

SUMMARY

Three cases have been reported in which a malignant tumor arose from the chromophobe cells of the pars anterior of the pituitary body. These differed clinically from the usual case of benign chromophobe

adenoma in the history of rapid progression of symptoms and in the rapid extension of the tumor into the skull bones adjacent to the sella turcica, the neighboring brain substance and the nasopharynx. The early stages of the illness were dominated in the first case by ocular symptoms, in the second by uncinate seizures and in the third by nasal obstruction.

Histologically, the tumors were characterized by arrangement of the tumor cells in broad sheets separated from one another by a stroma which was altered in character from that of the normal pars anterior and was in part derived from structures at the edge of the tumor, far from the sella turcica.

Such tumors should be set apart from the usual chromophobe adenomas and designated in a distinctive way. The term "malignant chromophobe adenoma" indicates that they are locally invasive and possess certain of the histologic characteristics of malignant tumors but do not metastasize either in the cerebrospinal axis or elsewhere in the body.

The malignant chromophobe adenoma presents certain special difficulties in differential diagnosis, especially in distinguishing it from chordoma and carcinoma of the sphenoid sinus or of the nasopharynx.

The tendency of the malignant chromophobe adenoma to include large blood vessels in the sellar region makes surgical approach to it especially perilous.

One of the 3 patients showed a very satisfactory response to biopsy followed by roentgen therapy, including the control of symptoms pointing to involvement of the temporal lobe. The other 2 patients, who were treated surgically, died.

NEURONOPHAGIA IN THE HUMAN CEREBRAL CORTEX IN SENILITY AND IN PATHOLOGIC CONDITIONS

WARREN ANDREW, PH.D.
DALLAS, TEXAS
AND
EDWARD SINTON CARDWELL JR., M.D.
AUGUSTA, GA.

A study has been made of the cerebral cortex of man in order to determine whether a process of neuronophagia occurs there which is as well defined as that reported recently [1a] in the brain of the mouse as a result of starvation to the point of inanition, and in senility.

An increase in satellitosis and a process of "neuronophagia" have been described by numerous workers with human tissue in a variety of pathologic conditions, but the process of actual ingestion of the neuron by the glial cells has not been described, nor are clear illustrations of this process available.

The changes in the Purkinje cells of the cerebellum in the mouse from youth to senility have been reported in an earlier paper.[1b] An attempt to compare the changes in the nerve cells of rodents and of man during the aging process was made in a study of human Purkinje cells in 34 autopsies on persons ranging in age from newborn to 83 years.[1c] Briefly, in old age the Purkinje cells of the mouse and of man showed a decrease in the amount of Nissl material, a change from the "clear" type of nucleus to the "basophilic" type and a loss of the smoothness of contour of the cell body.

Observations on the pyramidal cells of the cerebral cortex in the senile mouse [1a,d] have shown a decrease in Nissl material, a change from the "clear" to the "basophilic" property of the nucleus in these cells, and instances of apparent neuronophagia, an actual ingestion of the nerve cell by the glial cells.

It has been, however, the demonstration of active neuronophagia in the cerebral cortex of mice starved to inanition [1a] which has made it

From the Department of Anatomy, Baylor University College of Medicine, Dallas, Texas (Dr. Andrew), and the Department of Pathology, University of Georgia School of Medicine, Augusta, Ga. (Dr. Cardwell).

1. Andrew, W.: (*a*) J. Comp. Neurol. **70**:413, 1939; (*b*) Ztschr. f. Zellforsch. u. mikr. Anat. **25**:583, 1936; (*c*) **28**:292, 1938; (*d*) Am. J. Anat. **64**:351, 1939.

possible to describe this process as one of lysis and ingestion of the nerve cell and further has enabled us to recognize the stages in the process in senile mice and in the present study on human material.

MATERIALS AND METHODS

In the present investigation sections of human cerebral cortex and of cerebellum from 25 persons have been studied. The causes of death of these persons were varied, but it has been hoped that differences due to the specific pathologic condition of each person at the time of death would be overridden by the use of tissues from a large number of persons. Previous work with the Purkinje cells had tended to show also that the age of the subject is usually a more important factor in determining the appearance of the nerve cells than is the particular pathologic condition causing, or contributing to cause, death.

An attempt was made to avoid grossly abnormal areas, and when practical the right cerebral hemisphere was used, in order to insure uniformity. In all but a few of the cases two sections were taken from the cerebral cortex: one from the motor area and the other from the occipital lobe. The specimens of cerebellum were taken from the lateral hemispheres. All material was fixed in solution of formaldehyde U. S. P. (1:10), usually within three to seven hours after death. The tissue was then dehydrated in the alcohols, cleared in xylene, infiltrated, and embedded in paraffin blocks. In all cases a detailed gross and microscopic examination was made of the remaining viscera.

The blocks of tissue from the frontal cortex, occipital cortex and cerebellar cortex were sectioned at 8 microns and mounted on slides. The sections from 4 persons, usually of widely varying ages, were carried through the process of staining in the same glass slide tray, constituting a "series," in which a direct comparison as to staining qualities of the cells is readily made. All material was stained in a 1.5 per cent aqueous solution of cresyl violet for a period of seventy-five minutes.

Opinions as to the staining properties of the cytoplasm and nuclei, the shape of the cell body and the amount of satellitosis could be formed by a more or less superficial observation of the tissue under low power. It was felt, however, that a quantitative investigation was much to be desired.

For this purpose 100 cells from each region of each subject studied were carefully examined under the oil immersion lens (N.A. = 1.8) of a Bausch and Lomb monobjective binocular microscope (magnification, 970). These cells were classified according to the amount of Nissl material in the cytoplasm, the staining quality of the nucleus and the number of satellites in contact with the cell body.

Stages in neuronophagia and the relative frequency of their appearance were noted and recorded.

The data on the degree of satellitosis were obtained by a count of the glial cells in contact with the body of each nerve cell. Both microglial and oligodendroglial cells were included in this count, and a differentiation was not attempted, as the relative roles of the two types are still obscure and their distinction with Nissl staining probably not accurate. Only cells in contact with or encroaching on the body of the nerve cell were counted. Those touching the dendrites or axons were not included.

The method of making an observation on the cerebral cortex consisted in focusing on a particular part of the section from the region being studied, classifying the large pyramidal cells in this field as to number of satellites, then

passing to the immediately adjacent region in a direction parallel to the surface of the gyrus, classifying the large pyramidal cells here, and continuing in this way until 100 cells had been classified. In the cerebellar cortex we simply followed the outline of the granular layer until 100 Purkinje cells had been observed and classified as to the amount of Nissl material and the staining properties of the nucleus.

OBSERVATIONS

A study of the layers of the cerebral cortex reveals that definite neuronophagia is easily demonstrable in many of the specimens, and

TABLE 1.—*Material of Investigation**

Subject	Age, Years	Race	Sex	Cause of Death
1	7	Negro	♂	Acute rheumatic fever
2	12	White	♂	Anterior poliomyelitis
3	19	Negro	♂	Transverse myelitis (fractured cervical vertebra)
4	20	Negro	♀	Sickemia
5	25	Negro	♀	Hemorrhagic meningitis following mapharsen
6	35	Negro	♂	Cerebral hemorrhage
7	38	Negro	♂	Hypertensive nephritis; uremia
8	38	Negro	♂	Tuberculoma of pons; tuberculous meningitis
9	40	Negro	♂	Hypertension and cerebral hemorrhage
10	43	Negro	♀	Acute pulmonary edema (pneumonic)
11	45	White	♂	Acute alcoholism
12	45	White	♂	Dementia paralytica
13	46	Negro	♂	Dementia paralytica
14	48	White	♀	Old cerebral infarct
15	49	Negro	♂	Traumatic injury of brain
16	55	Negro	♂	Cerebral hemorrhage
17	56	White	♂	Cerebral thrombosis
18	60	White	♂	Cerebrospinal syphilis
19	63	Negro	♂	Lymphogranulomatous stricture of rectum
20	65	White	♂	Cardiac failure
21	67	Negro	♂	Cerebral hemorrhage; hypertension
22	69	White	♂	Bacterial endocarditis with embolic infarcts of meninges, spleen and kidneys
23	70	Negro	♂	Coronary occlusion
24	75	Negro	♀	Hypertensive nephritis; uremia
25	86	Negro	♂	Traumatic pneumonia

* The subjects from whom brain tissue was obtained at autopsy are numbered consecutively in the first column in the order of age at the time of death. In the second, third and fourth columns are given the age, race and sex of each subject; for instance, 7, Negro, ♂ indicates a 7 year old Negro male; 45, white, ♂, a 45 year old white male. In the fifth column is given the cause of death as listed in the autopsy charts.

with some searching, in the majority of them. It is in the layer of polymorphic cells, just below the layer of large pyramidal cells, that the most active phagocytosis is seen to occur.

In specimens 1 to 8, inclusive, we find neuronophagia infrequently and only after diligent search. In these specimens the process, when seen, is similar in all respects to the process as we have studied it in young mice which have been starved to inanition. It consists in an encroachment by the glial cell, usually a solitary cell, on the substance of the neuron, in a widening crescent, the body of the glial cell growing as the substance of the nerve cell disappears and is ingested. In specimen 8 it is possible to find more than one stage of the process in a single field among the polymorphic cells, under the oil immersion lens

(fig. 1 *B*). By proper focusing the actual outline of the clear cell body of the glial cell can be made out quite readily in these Nissl preparations.

In man, as in the mouse, the nucleus of the nerve cell appears to be the most resistant cell organ and often remains, enclosed in a thin crescent of cytoplasm, when nearly all of the cell body has been phagocytosed.

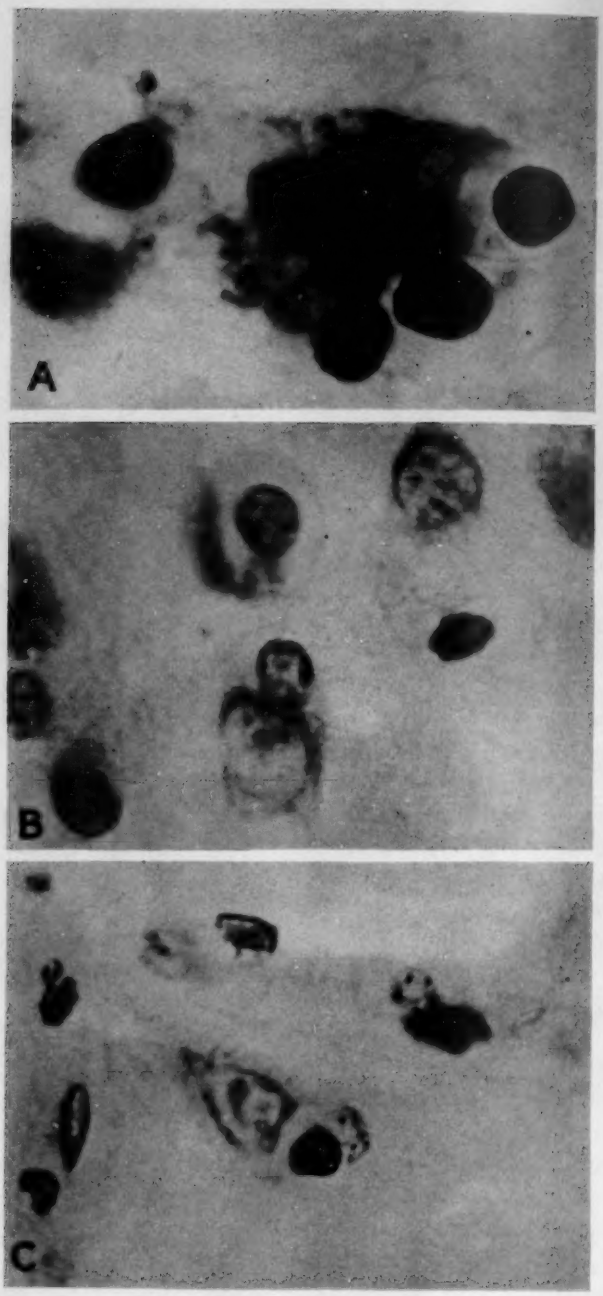
The process here, as in the mouse, appears to be essentially one of lysis followed by ingestion. However, in the end stages, as in the cell in the upper part of figure 1 *B*, there is sometimes at least an appearance as though the glial cell had engulfed the remaining formed portions of the neuron without lysis.

In specimens 9 to 18, inclusive, neuronophagia is found considerably more frequently than in the preceding specimens. In these specimens the nerve cells more often are being phagocytosed by a group of glial cells, encroaching on the neuron from all sides. There are, however, still many instances in which only 1 or 2 glial cells are taking part in the process.

In specimens 19 to 25, inclusive, neuronophagia is much more abundant than in the aforementioned specimens. In some of these specimens, particularly in 21 and 25, often several instances of neuronophagia may be seen in a single field in the layer of polymorphic cells, under the oil immersion lens. In these 7 specimens the neurons being phagocytosed almost invariably are being attacked by several glial cells, which sometimes form a ring about the cell body and at other times encroach on it predominantly from one side or the other. In figure 1 *A* a nerve cell is being attacked by such a group of glial cells, 3 of the cells destroying the cell body on one side, a single cell acting on the other.

Among the nerve cells being phagocytosed in any of the age groups described there are large numbers which have by no means lost their typical structure. The nucleus and nucleolus may remain apparently intact even until the cell body has vanished almost completely, and a nerve cell in process of being destroyed frequently shows definite Nissl bodies.

The chief differences in the amount of neuronophagia occurring in the layer of polymorphic cells and in the type occurring, i. e., whether 1 or 2 or a group of 3, 4 or 5 glial cells are active in the phagocytosis of a single neuron, are more closely correlated with age than with any of the other differences among the 25 subjects. These age differences resemble those seen in the mouse. In the brains of young healthy mice neuronophagia is not seen. When, however, neuronophagia is induced by starvation to the point of inanition, the process of ingestion of a whole neuron usually is accomplished by a single glial cell. In senile animals, adequately fed, neuronophagia can be found in many instances



EXPLANATION OF FIGURE 1

A, a nerve cell from the layer of polymorphic cells of the cerebral cortex of an 86 year old man, specimen 25, in process of being phagocytosed by four glial cells, the outlines of which can be seen clearly. Nissl preparation; × 2,500.

B, two nerve cells from the layer of polymorphic cells of the cerebral cortex of a 38 year old man who died of widespread tuberculosis (specimen 8). The cell above has been phagocytosed almost completely, and the large, "swollen" body of the glial cell can be seen. The cell below has been only partially phagocytosed, the nucleus and nucleolus being still visible. Nissl preparation; × 1,000.

C, nerve cell from the polymorphic layer of the cerebral cortex of the same subject (specimen 8). The cell is partially phagocytosed by a glial cell, the clear cell body of which can be seen invading the cytoplasm of the neuron. Nissl preparation; × 1,000.

and is of the type in which several glial cells attack a single neuron. In starved senile animals this process is greatly accentuated, but in nearly all cases several glial cells attack a single nerve cell.

Neuronophagia was found to be far less active in the layers superficial to the layer of polymorphic cells. Satellitosis is present as a normal feature in the layer of large pyramidal cells but in those brains in which neuronophagia was very abundant, the degree of satellitosis, as judged by low power observation, was definitely increased. Instances of actual phagocytosis of the large pyramidal cells in such brains could

TABLE 2.—*Data on Large Pyramidal Cells of the Motor Cortex*

Subject	Age, Years	100 Nerve Cells Grouped According to Number of Glial Satellites*						Total Number of Satellites per 100 Cells
		0	1	2	3	4	5	
1	7	74	19	6	1	0	0	34
2	12	Motor cortex not obtained						
3	19	75	18	5	2	0	0	34
4	20	69	24	6	1	0	0	39
5	25	73	20	5	2	0	0	36
6	35	64	30	4	2	0	0	44
7	38	72	18	10	0	0	0	38
8	38	74	18	2	1	1	0	35
9	40	65	25	5	4	0	1	52
10	43	63	19	11	7	0	0	62
11	45	50	31	12	4	3	0	79
12	45	76	16	7	1	0	0	33
13	46	60	28	11	1	0	0	53
14	48	Motor cortex not obtained						
15	49	73	18	5	4	0	0	40
16	55	64	30	4	2	0	0	44
17	56	Motor cortex not obtained						
18	60	78	16	4	2	0	0	30
19	63	78	28	11	1	0	0	26
20	65	61	28	8	2	1	0	54
21	67	58	24	13	5	0	0	65
22	69	61	29	8	2	0	0	51
23	70	58	26	11	4	1	0	64
24	75	46	27	16	6	4	1	98
25	86	63	26	9	2	0	0	50

* Under the heading "100 Nerve Cells Grouped According to Number of Glial Satellites" there are found in the "0" column the number of nerve cells with no satellites, in the "1" column the number with one satellite, and so on.

be found, but were many times less frequent than among the polymorphic cells.

It was thought desirable to obtain some quantitative check on the degree of satellitosis. In tables 2 and 3 are given the data on the large pyramidal cells classified as to the number of glial satellites in contact with them. A study of the tables shows, in spite of the considerable degree of variation, that there is a marked increase in the average amount of satellitosis in the older persons as compared with the younger. Thus in the motor cortex (table 2) in the 7 persons under 40 years of age the average number of satellites found in contact with the bodies of 100 large pyramidal cells studied is 37. In the 8 persons

of ages from 40 to 60 years, inclusive, the average number of glial satellites is 49. In the 7 persons of ages 63 to 86 years, inclusive, the average number is 58.

In the occipital cortex the average number of satellites in persons under 40 years of age is 39; in those from 40 to 60 years, inclusive, it is 44, and in those from 63 to 86 years, inclusive, it is 57. It does not seem profitable to attempt to explain the individual differences in the degree of satellitosis among persons of the same age group, but we do see in both frontal and occipital cortex a definite increase in the degree

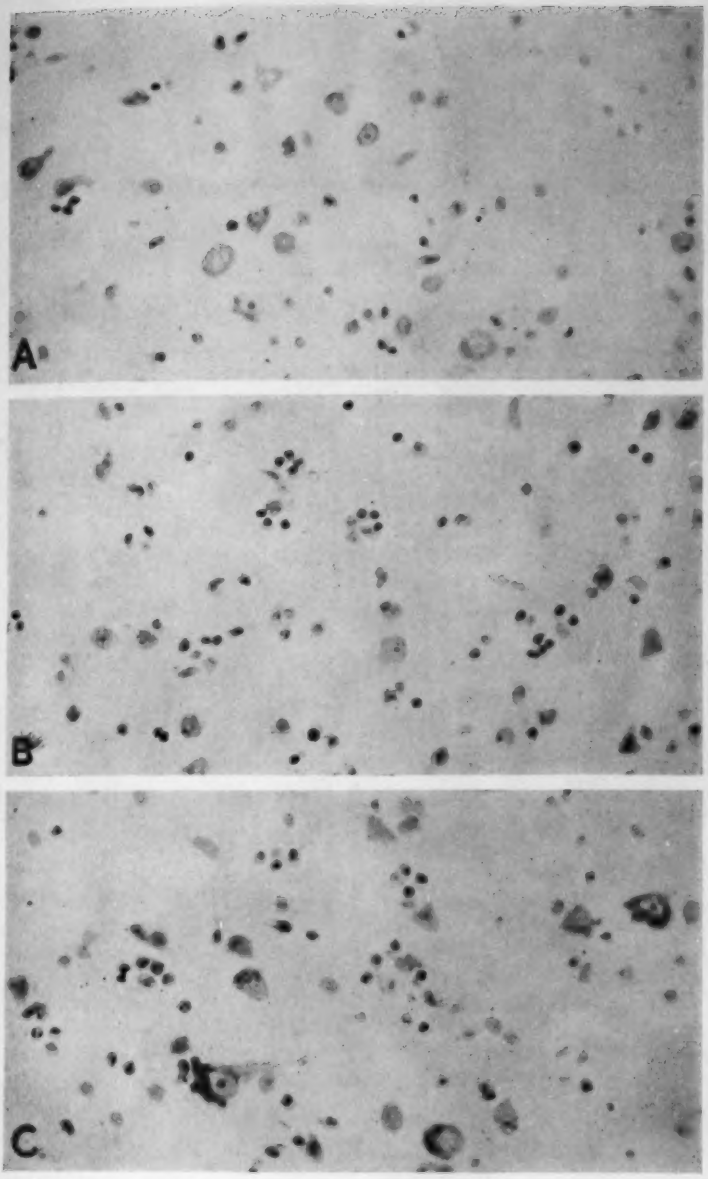
TABLE 3.—*Data on Large Pyramidal Cells of the Occipital Cortex*

Subject	Age, Years	100 Nerve Cells Grouped According to Number of Glial Satellites*						Total Number of Satellites per 100 Cells
		0	1	2	3	4	5	
1	7	72	25	3	0	0	0	31
2	12	79	15	6	0	0	0	27
3	19	79	18	1	2	0	0	23
4	20	69	24	6	1	0	0	39
5	25	69	23	11	1	0	0	40
6	35	74	20	4	2	0	0	34
7	38	69	24	5	2	0	0	40
8	38	49	33	14	3	1	0	74
9	40	Occipital cortex not obtained						
10	43	60	29	8	3	0	0	54
11	45	Occipital cortex not obtained						
12	45	65	23	9	2	1	0	51
13	46	69	22	5	1	1	0	39
14	48	65	23	11	1	0	0	48
15	49	60	19	16	5	0	0	66
16	55	79	14	6	0	1	0	30
17	56	80	14	5	1	0	0	27
18	60	69	24	7	0	0	0	38
19	63	68	20	9	2	1	0	52
20	65	Occipital cortex not obtained						
21	67	46	30	17	5	2	0	87
22	69	78	21	0	1	0	0	24
23	70	63	24	7	5	1	0	57
24	75	Occipital cortex not obtained						
25	86	55	30	10	4	1	0	66

* Under the heading "100 Nerve Cells Grouped According to Number of Glial Satellites" there are found in the "0" column the number of nerve cells with no satellites, in the "1" column the number with one satellite, and so on.

of satellitosis with advancing age in these persons. It seems probable that this increase in satellitosis is definitely related to the increase in neuronophagia.

The amounts of Nissl material in the large pyramidal cells of the cerebral cortex and in the Purkinje cells of the cerebellum show a very large degree of difference among persons even of very nearly the same age, as we might expect in dealing with such varied pathologic conditions. However, with a single very notable exception, the senile persons show a relatively very large number of hypochromatic cells. The exception is the oldest specimen of all, that from an 86 year old Negro, in which the great majority of the nerve cells are chromatic. The body of this man was described as having been in excellent nutritional and



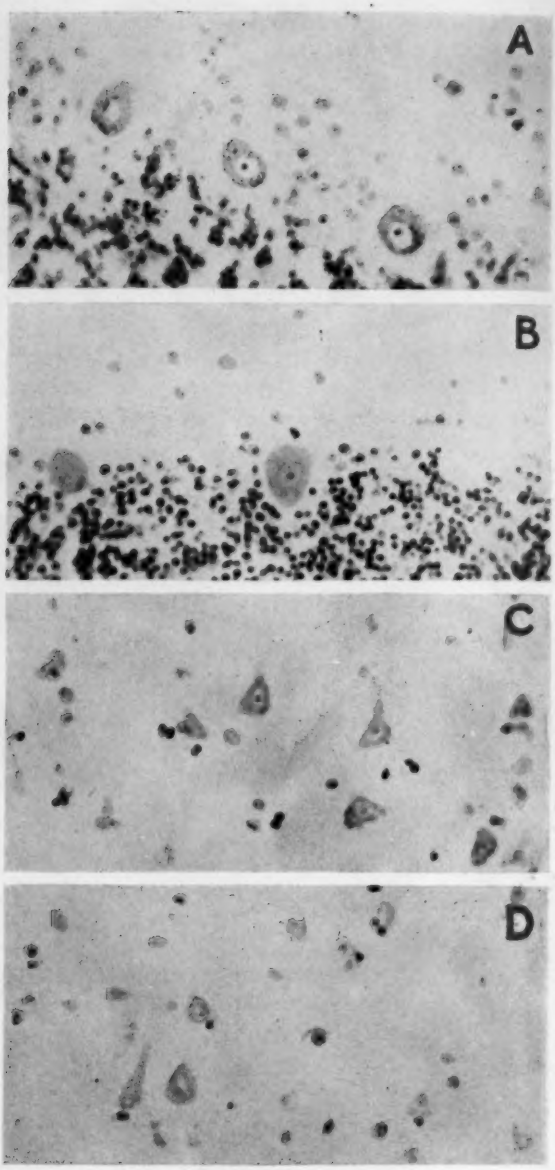
EXPLANATION OF FIGURE 2

A, *B* and *C* are photomicrographs from Nissl preparations; × 250.

A, layer of polymorphic cells of the cerebral cortex of a 19 year old man (specimen 3). The nerve cells are essentially normal. Satellitosis is not marked, and neuronophagia is rare.

B, layer of polymorphic cells of the cerebral cortex of a 67 year old man (specimen 21). Satellitosis is marked, and neuronophagia is common.

C, layer of polymorphic cells of the cerebral cortex of an 86 year old man (specimen 25). Satellitosis is common and neuronophagia abundant.



EXPLANATION OF FIGURE 3

A, *B*, *C* and *D* are photomicrographs from Nissl preparations; × 250.

A, Purkinje cells of a 12 year old boy (specimen 2). The Nissl granules are abundant and the nuclei clear.

B, Purkinje cells of a 75 year old woman (specimen 24). There is little or no Nissl material in these cells, and the nuclei are basophilic.

C, layer of large pyramidal cells of a 19 year old man (specimen 3). The cells contain considerable Nissl material.

D, layer of large pyramidal cells of a 75 year old woman (specimen 24). A large proportion of the cells are hypochromatic.

muscular condition at the time that it came to autopsy. Whether this general condition may be the explanation of the condition of the nerve cells, we cannot say. Certainly it has been shown that it is possible for a senile person to have a majority of nerve cells with abundant Nissl material.

There is an increasingly larger number of nuclei with basophilic staining properties in the older persons. To this statement, our oldest subject is, however, again an exception.

In the majority of the subjects the relative numbers of chromatic and hypochromatic cells seen in the motor area, in the occipital cortex and in the cerebellum correspond fairly closely. Thus the condition of cells throughout the brain at the time of autopsy seems to be very similar in any given person.

No evidences of neuronophagia are seen in the cortex of the cerebellum nor is there any increased satellitosis about the Purkinje cells in senile persons.

COMMENT

The most important conclusions from the present study are that neuronophagia occurs in the cerebral cortex of man and that the process is essentially similar to that which is induced in the mouse by starvation. Neuronophagia has been found to some degree in the layer of polymorphic cells and to a lesser extent in the more superficial layers of the cortex in the majority of the pathologic specimens examined. The most abundant neuronophagia was found, however, in the senile brains.

We have been unable to find any earlier work in which a clear demonstration of the process of neuronophagia in the human cerebral cortex has been made. Glial proliferation in the senile brain has, however, been noted by several other workers. The question which has not been answered by the earlier workers appears to be what such glial proliferation indicates, whether it represents merely a substitution, the glia taking the place of the degenerating nerve cells, or whether the glial cells are taking an active part in the removal of these nerve cells, perhaps even in the destruction of living nerve cells.

Both the earlier and the more recent authors have noted glial proliferation in the senile brain, and some of them have spoken of an increased degree of satellitosis. Many of these authors, however, have concluded that this is purely a substitution or have not attempted to interpret its significance.

Recent papers, such as that by Cardona,[2] have reaffirmed the importance of glial changes as a criterion of pathologic changes in the human brain.

2. Cardona, F.: Riv. di pat. nerv. **50**:141, 1937.

Gellerstedt,[3] who made an extensive study of senile brains, apparently concurred with earlier workers who did not look on increased satellitosis as indicative of neuronophagia. He stated (p. 332): *Diese "Phagozyten," besonders der tieferen Rindenschichten, sind von Saigo 1907 als eine senile Trabantenzellwucherung erkannt worden* (These "phagocytes," especially those of the deeper cortical layers, were recognized by Saigo in 1907 as a senile proliferation of satellite cells). He seems to have agreed with the earlier author that these cells probably are not phagocytes but simply satellites. Gellerstedt did find that the progressive changes in glial cells, rather than the regressive, are characteristic of the senium (ibid., p. 401): *Die regressive Gliazellveränderung gehört nicht zum senilen Bilde* (The regressive changes in the glial cells do not belong to the senile picture).

Belezky [4] also found an increased number of mesoglial cells without processes and with few processes (oligodendroglia) both in normal senility and in senile psychoses.

Gellerstedt [3] found also a definite parallelism between the degree of glia proliferation and the degree of atrophy of the brain, even in specific localities in the brain. Nevertheless, he evidently thought of neuronophagia rather as a conception than as a demonstrable process. Thus he wrote (p. 336): *Das Eindringen von Mikroglia in die Drusen der Hirnrinde war natürlich auch in unsrem Material kein seltenes Ereignis ebensowenig, auf das Gesamtmaterial bezogen, das Auftreten von "Neurono"- und Gliophagien, vor allem im Hirnstamm und Kleinhirnmark* (The penetration of microglia into the drusen of the cerebral cortex was of course likewise not a rare occurrence in our material, just as little, in reference to the whole material, as was the appearance of neuronophagia and gliophagia, especially in the brain stem and in the cerebellar medulla).

Mühlmann [5] made a study of the glia in the human brain at various ages, but did not speak of the occurrence of neuronophagia nor even of any markedly increased degree of satellitosis.

Einarson and Okkels [6] spoke of neuronophagia as occurring in the cerebral cortex and in other parts of the brain of a 93 year old woman but did not describe or figure the process in their paper.

Schükrü-Aksel,[7] in his study of the brain of "the oldest man in the world," found great numbers of a new type of large glial cell in the

3. Gellerstedt, N.: Upsala läkaref. förh. **38**:193, 1933.

4. Belezky, W. K., and Jermolenko, E. I.: Virchows Arch. f. path. Anat. **291**:607, 1933.

5. Mühlmann, M., and Mutalimow, B.: Anat. Anz. **80**:218, 1935.

6. Einarson, L., and Okkels, H.: Ann. d'anat. path. **13**:557, 1936.

7. Schükrü-Aksel, J.: Arch. f. Psychiat. **106**:260, 1937.

cerebral cortex, basal ganglions, mesencephalon and dentate nucleus. He wrote (p. 263): *Merkwürdig ist aber das Hervortreten eigenartiger Gliakerne, die an Pseudosklerosezellen errinern. Diese Gliakerne sind dreimal so gross wie die normalen* (Noteworthy, however, is the emergence of peculiar glial nuclei, which recall pseudosclerosis cells. These glial nuclei are three times as large as the normal ones). He did not describe these as showing a tendency toward satellitosis nor did he describe an increase in satellitosis by the ordinary, smaller glial cells except in certain regions. He did say of the hypothalamus (p. 265): *Man findet hier aber Trabantzellenansammlungen um die Nervenzellen herum* (One finds here, however, accumulations of satellite cells around the nerve cells).

Rothschild [8] said of the cases of senile psychoses which he studied (p. 765): "Several cases were observed in which especially severe cell losses combined with considerable neuroglial proliferation produced outspoken disturbances of the cyto-architecture."

We see, then, that many authors have observed proliferation of glia in the senile brain, that some have noted increased satellitosis and that a few have spoken of neuronophagia as a function of the glial satellites. We feel that as a result of the present study we can without hesitation affirm that neuronophagia is occurring actively in the brains of the majority of senile persons. We believe also that the increase in satellitosis is definitely related to the process of phagocytosis. How long the destruction of a neuron in the senile brain may take, it is as yet impossible to say, but the indications are that the process may be a very slow one, certainly as compared with that in starved animals.[1a]

The process seen in these senile human brains appears to be essentially similar to that previously studied in the mouse. It consists in lysis and ingestion of the neuron by the glial cells, 1 to 5 or even more of these cells taking part in the phagocytosis of a single large neuron. Here, as in the mouse, the nucleus is usually the last of the cell parts to be ingested.

In man, as also in the mouse, the Purkinje cells appear to be wholly immune to any phagocytic action by the glial cells. We have yet to see a single instance of an attack on these cells. The only author whom we have found to speak of any increased degree of satellitosis or of phagocytosis of Purkinje cells is Marinesco,[9] who stated that such phenomena might be observed after injection of bile into the cerebellum.

The loss of Nissl material in the cells of both the cerebral cortex and the cerebellum was a feature of the senile brains studied in this investigation with the notable exception of the oldest of all, that of the

8. Rothschild, D.: Am. J. Psychiat. **93**:757, 1937.

9. Marinesco, G.: Ann. d'anat. path. **7**:341, 1930.

86 year old Negro. Predominance of hypochromatic cells in the cerebral cortex of a woman dead of senility at 90 years of age was reported by Robertson and Orr.[10] Salimbeny and Géry [11] reported increased acidophilia in the ganglion cells, especially in the Purkinje cells, of an 83 year old woman, together with irregularity of cell outline, atrophy, and degenerative changes in the nucleus. Ellis,[12] in his quantitative study of the loss of Purkinje cells with advancing age, found degenerative changes in these cells in senility, including chromatolysis and atrophy.

Einarson and Okkels [6] found that chromatolysis was a conspicuous feature in the brain of a 93 year old woman.

Rothschild [8] in a detailed study of 24 cases of senile psychoses found many cells with pale, ill defined Nissl bodies, and in the more severe cases the Nissl material was altered to a dustlike material uniformly scattered throughout the cytoplasm. He also found cell shadows, or "ghosts," to be common. He said (p. 782): "Equally severe alterations may be found in the brains of old persons of normal mentality."

The author of the most extensive work on changes in the brain in normal senile involution (Gellerstedt [3]) has, in spite of the findings by the other authors mentioned and by others not mentioned, taken the view that hypochromatism of large numbers of cells may not be a feature of normal senility, or that in life the normal senile brain may contain perhaps as many chromatic cells as the brain of a younger person and that the greater loss of Nissl material from the cells in senile persons may be due simply to the greater vulnerability of the cells in such persons to the damages wrought in the final stages just before death. He said of the hypochromatic cells (p. 270):

> Solche waren nun in den verschiedensten Hirnregionen unsres Materials, und zwar in jedem Falle, zu sehen, gar nicht selten aber auch im jüngeren und jüngsten Kontrollmaterial gesunder wie kranker Individuen. Verf. ist also eher geneigt, *ihren Charakter als typische senile Veränderung zu bestreiten und an präagonale Absterbeerscheinungen bei Ganglienzellen zu denken.* Dass sie jedoch im Senium so besonders häufig sind, könnte mit der grösseren Vulnerabilität alternder Zellen zusammenhängen (Such were to be seen in the most diverse cerebral regions of our material, and in every case, and not rarely also in the younger and youngest control material of healthy as well as of diseased individuals. The author is therefore rather inclined to deny their character as typical senile changes and to think of them as preagonal manifestations of the dying process in ganglion cells. That they are so frequent during senility would be due to the greater vulnerability of aging cells).

Gellerstedt examined a large amount of material, the brains of 50 persons over 65 years of age, prepared according to numerous technics,

10. Robertson and Orr: J. Ment. Sc. **44**:729, 1898.
11. Salimbeny, A. T., and Géry, J.: Ann. Inst. Pasteur **26**:577, 1912.
12. Ellis, R. S.: J. Comp. Neurol. **32**:1, 1920.

and hence his conclusions should be worthy of serious consideration. We shall note, however, that he did find hypochromatic cells much more abundant in the senile material, and the question which he raised is: How did this greater abundance come about?

We cannot wholly agree with his answer to this question. We believe that the large number of hypochromatic cells is a characteristic of the average senile brain and not due to changes just before death. Support for this standpoint is derived from a comparative study of the brains of animals other than man, killed in a variety of ways. In all of the work of one of us (W. A.) on the mouse [1a,b,d] the loss of Nissl material from the nerve cells is a conspicuous feature of the brain of the senile animal. The great majority of the animals were killed by decapitation and the brain removed immediately and placed in fixative. The time from decapitation until the brain was in fixative was never more than three or four minutes, and it is thought unlikely that such striking loss of Nissl material as is seen would occur in so short a time. Other authors (Dolley,[13] on the dog, Inukai,[14] on the rat) have found increased numbers of hypochromatic cells in the brains of senile specimens among the lower animals.

We believe that the higher number of hypochromatic cells in the brains of older men and women speaks for the loss of Nissl material as a phenomenon of the aging process and as a gradual rather than a rapid or sudden process.

It does not seem surprising that there should be considerable variation in the degree of loss of Nissl material at any particular age when we consider the differences in other characteristics of persons in the same age group. An interesting example of this is seen in the report by Schükrü-Aksel [7] on the brain of the "oldest man in the world," Zaro Aga, who, according to reliable data, must have been about 130 years of age at the time of his death. Schükrü-Aksel wrote (p. 263): *Die Ganglienzellen enthalten ziemlich gut angeordnete Nissl-Schollen* (The ganglion cells contain rather well arranged Nissl material). The 86 year old man in our series showed, as we have said, abundant Nissl material. This is, however, we believe, an exception to a rule. In this man's brain neuronophagia was very common, as in the other senile brains.

The change in the staining properties of the nucleus which leads to a great decrease in the number of nuclei of clear type and an increase in those of basophilic type with increasing age is a phenomenon which had been noted previously in the mouse [1a,b,d] and in the Purkinje cells of man. We have been unable to find this phenomenon described

13. Dolley, D. H.: J. M. Research **24**:309, 1911.

14. Inukai, T.: J. Comp. Neurol. **45**:1, 1928.

clearly as an accompaniment of aging of the nerve cell in the papers of earlier workers, although there are some references to larger numbers of hyperchromatic nuclei in senile persons as in the paper by Einarson and Okkels [6] on the brain of an 83 year old woman.

Butler,[15] in reporting on changes in depression of the nerve cell produced by long ether anesthesia, said (p. 330): "In depression the nucleus contains increased amounts of nuclear materials, both chromatin and nucleolar substance, corresponding to the degree of depression, while the plasma is deficient in chromatin (Nissl substance)."

Among all the Purkinje cells examined in human senile brains no cells with double nuclei nor any showing amitotic divisions of the nucleus have been found, although such cells are encountered frequently in the brains of senile mice.

We wish to call attention to the presence of pigment in almost 50 per cent of the Purkinje cells in the brain of one of the senile subjects, no. 24. In this subject many of the large pyramidal cells also showed pigment. Dolley [16] said concerning abnormal pigmentation in the nervous system (p. 482): "It is a phenomenon of chronic depression, or depressant senility." Harms [17] found much pigment present in the ganglion cells from many parts of the nervous system of a woman 80 years of age.

We have not, however, found pigmentation as a constant feature in the senile brains which we have examined.

SUMMARY

Neuronophagia, a process of lysis and ingestion of the nerve cell, as demonstrated in the mouse,[1a] occurs in the human cerebral cortex in senility and in a number of pathologic conditions. This process is most active in the layer of polymorphic cells.

There is an increase in the degree of satellitosis about the large pyramidal cells with advancing age.

A loss of Nissl material and an increase in the basophilic properties of the nuclei are found in most of the older persons and are believed to be natural phenomena of aging in man.

15. Butler, E. E.: J. M. Research **34**:325, 1916.
16. Dolley, D. H.: J. Comp. Neurol. **28**:465, 1917.
17. Harms, J. W.: Zool. Anz. **74**:249, 1927.

Case Reports

INSULIN HYPOGLYCEMIA AND GLYCOGENIC HEPATOMEGALY IN DIABETES MELLITUS

NATHAN B. FRIEDMAN, M.D., CHICAGO

Although the observation of increased amounts of glycogen in both the normally sized and the enlarged liver of the diabetic patient is an old one, it may be of value to record it again since the emphasis in recent studies has been on the deposition of fat in such livers.[1] The finding of large glycogen-laden livers has led some workers to the consideration of such a condition, even when it occurs in a patient with undoubted diabetes, as belonging to the category of glycogen storage disease (Von Gierke's disease).[2] Transitions from glycogen disease to diabetes are also recorded.[2b]

It is not the purpose of this paper to deny the existence of these transitional or mixed forms but to reemphasize the fact that glycogen storage may be a prominent feature of diabetic hepatomegaly. The cases to be reported illustrate hepatomegaly associated with insulin hypoglycemia in diabetic patients, and in these cases the hepatomegaly was due to storage of glycogen rather than to storage of fat.

REPORT OF CASES

CASE 1.—(This case is reported with the permission of Drs. Joseph Brennemann and William G. Hibbs.)

A 3½ year old boy was admitted to the Children's Memorial Hospital (service of Dr. Alvah Newcomb) in coma of one day's duration after three weeks' loss of weight, polydipsia and polyuria. He was comatose, dehydrated and breathing deeply and had an acetone breath. The temperature at its highest was 101 F. The blood pressure was 112 systolic and 66 diastolic. There were a few rales in the lungs and a barely palpable liver.

The urine showed sugar (4 plus) and acetone, and the blood sugar was 500 mg. per hundred cubic centimeters.

He received insulin in large doses and, parenterally, dextrose solution, saline solution and lactate–Ringer solution (as devised by Dr. Hartman). During each eight hour period following admission he received about 1,000 cc. of these fluids. The amount of insulin given in each of these eight hour periods was as follows: 180, 120, 330, 220 and 200 units (in the first three of these doses 120 units of protamine insulin are included). During the last eight hours of his life he received no insulin.

From the Department of Pathology of the University of Chicago.

1. (*a*) Warren, S.: The Pathology of Diabetes Mellitus, Philadelphia, Lea & Febiger, 1938. (*b*) Marble, A.; White, P.; Bogan, I. K., and Smith, R. M.: Arch. Int. Med. **62**:740, 1938. (*c*) Dragstedt, L. R.; Van Prohaska, J., and Harms, H. P.: Am. J. Physiol. **117**:175, 1936.

2. (*a*) Terplan, K.; Vogel, S., and Hyde, E.: Arch. Path. **26**:599, 1938. (*b*) van Creveld, S.: Medicine **18**:1, 1939.

The blood sugar fell at one time to 196 mg. and varied between this level and 500 mg. per hundred cubic centimeters. The carbon dioxide–combining power remained low, between 14 and 21 volumes per cent. Sugar persisted in the urine (4 plus) but acetone later disappeared.

He remained in semicoma and had two episodes of convulsions with rigidity of the extremities. Twelve hours before death the blood sugar was 285 mg. Three hours later spastic opisthotonos developed, and cerebral damage was suspected. A spinal tap showed increased pressure, and the spinal fluid contained 222 mg. of sugar per hundred cubic centimeters. Three hours later, however, the blood sugar was found to be 17 and one hour later 30 mg.

He died six hours later despite the fact that the administration of insulin had been discontinued and that five doses of 10 to 25 cc. of 50 per cent dextrose solution had been given intravenously in addition to the continuous infusion.

Pathologic Observations (Dr. W. Price Killingsworth).—The body weighed 26 pounds (11.5 Kg.) and was 37 inches (94 cm.) in length. The heart and kidneys appeared normal. The liver weighed 650 Gm. (normal, 400 to 500 Gm.[3]), was deep red and cut with resistance. The pancreas weighed 5 Gm. (normal, 17 to 18 Gm.[4]). The brain was congested and contained tiny focal meningeal hemorrhages.

Microscopic Observations.—The heart and kidneys were essentially normal. The thyroid and adrenals showed no changes.

The liver cells were markedly vacuolated and clear (*A* in figure). They were laden with glycogen and showed relatively little fat (*B* in figure).

The pancreatic acinar tissue appeared normal, but the islets were scarce. In the body and head the islets were practically absent, and only in the tail did they approach the normal size and number. Their cellular structure appeared to be normal.

Case 2.—A 22 year old man was admitted to the University of Chicago Clinics (service of Dr. Louis Leiter) in March 1935, with hematuria and a painful mass in the right side of the abdomen.

Diabetes had been discovered at the age of 15, and he had been followed in the aforementioned clinics thereafter. At the outset he had required 25 units of insulin daily, but later took from 40 to 60 units. In February 1934 he had a preauricular carbuncle and required up to 80 units daily.

Laparotomy two days after his admission revealed a large pyonephrotic right kidney with an aberrant artery across the ureter, and the kidney was removed. Following this a septic temperature developed, ranging to 103 F., and blood cultures yielded Staphylococcus aureus. There was also bilateral draining otitis media.

His daily intake (orally and by vein) varied considerably, ranging from 50 to 250 Gm. of carbohydrate, with a total caloric value of from 500 to 1,500. Although he was given from 40 to 120 units of insulin per day, the blood sugar remained high (201 to 470 mg. per hundred cubic centimeters), and there was glycosuria (to 2 per cent).

On the fifth postoperative day he had episodes of irrationality and struggling. Meningitis was suspected, but lumbar puncture gave negative results. On the twelfth postoperative day his intake had included 130 Gm. of carbohydrate (total calories, 600), and 60 units of insulin had been given. The urine contained no

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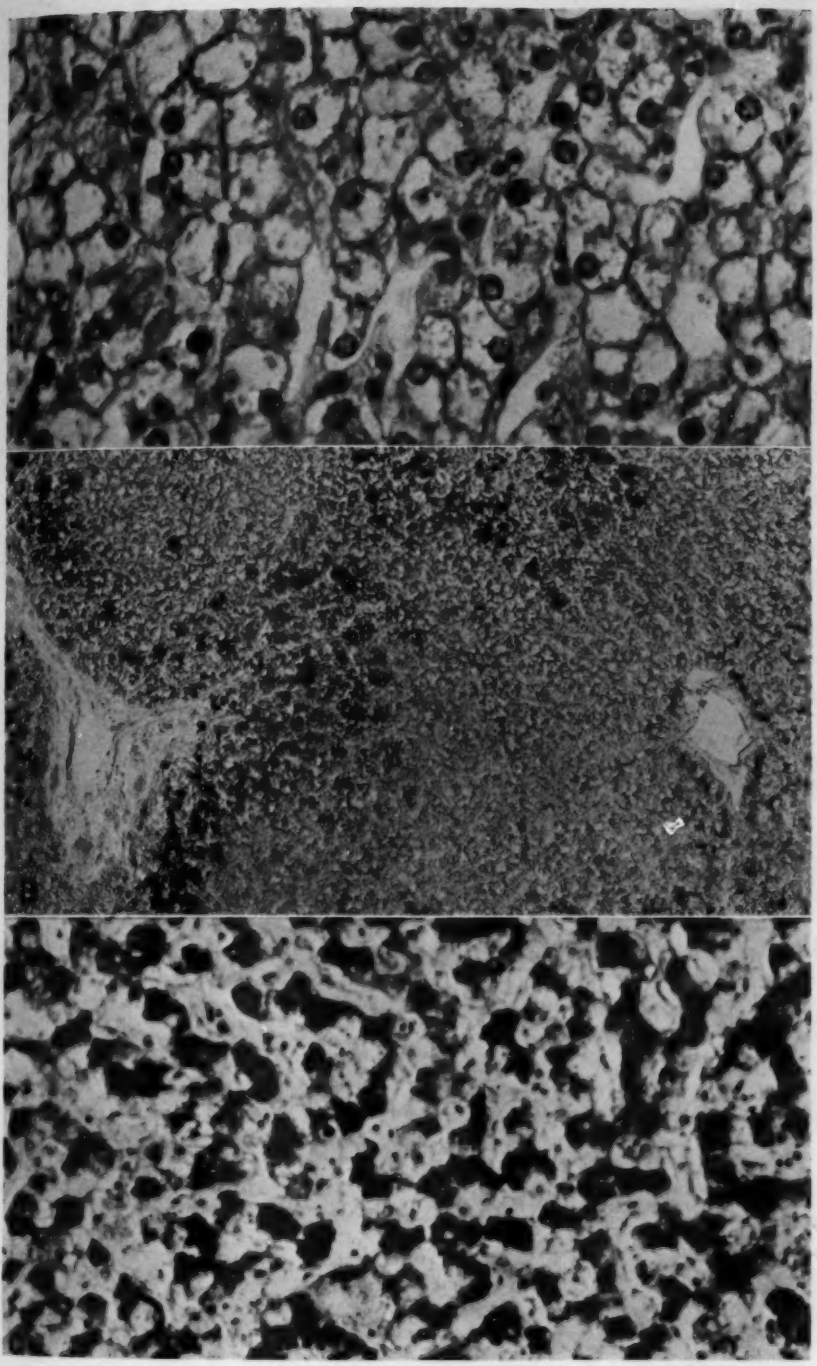
3. Coppoletta, J. M., and Wolbach, S. B.: Am. J. Path. **9**:55, 1933.

4. Gruber, G. B., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1929, vol. 5.



A, microscopic appearance of the liver in case 1. *B*, same liver stained for fat (sudan). *C*, liver in case 2 stained for glycogen (Best's carmine stain).

sugar. Then blurring of vision developed, with a positive Babinski sign, rigidity of the extremities and slowness of respiration with periods of apnea. On intravenous administration of 50 cc. of 50 per cent dextrose solution he regained consciousness, his muscles relaxed, and respiration became normal, all within five minutes. Between two and three hours later, 20 additional units of insulin having been given, a similar seizure occurred, and he died.

Pathologic Observations (Dr. Paul R. Cannon).—The body weighed 122 pounds (55 Kg.) and was 66 inches (167.5 cm.) in length. There were multiple abscesses in the remaining (left) kidney, thrombosis of the left renal vein, and abscesses in the myocardium, lungs and one epididymis. The heart weighed 280 Gm. The aorta was moderately atherosclerotic.

The liver weighed 3,100 Gm. (normal, 1,500 to 1,700 Gm.) and was smooth, pale and translucent. The pancreas weighed 47 Gm. (normal, 70 to 90 Gm.). The brain was edematous but revealed no meningitis.

Microscopic Observations.—There were some deposits of glycogen in the renal tubules. The thyroid appeared normal. One adrenal revealed a single small area of cortical atrophy.

The markedly vacuolated hepatic cells were laden with glycogen (*C* in figure) and contained practically no fat. The Kupffer cells were fatty. The pancreatic islets were small and not numerous, with the cells showing small dark nuclei and poorly defined scant cytoplasm.

COMMENT

The hepatomegaly in the 2 cases reported was associated with deposition of glycogen and may have been related to the insulin hypoglycemia.

Wohlwill [5] reported the presence of large amounts of glycogen in the liver of a diabetic patient who died in insulin hypoglycemia. Dupérie and Maupetit [6] described an insulin-treated boy of 7 with glycosuria and acidosis who died in coma and convulsions (hypoglycemic?) and showed a 1,030 Gm. liver rich in glycogen and containing only small amounts of fat.

Glycogenic hepatomegaly in a diabetic patient without hypoglycemia has been reported by Brian, Schechter and Parsons [7] as well as by Terplan, Vogel and Hyde,[2a] although the latter entitled their report "Prolonged Diabetes Mellitus Culminating in von Gierke's Disease in a Youth of Childlike Habitus."

Popper and Wozasek [8] reported several cases of diabetes in which the liver contained large amounts of glycogen. Kaufmann [9] stated that occasionally diabetic persons showed large collections of hepatic glycogen and that their livers were large and heavy. Warren [1a] described a 3,900 Gm. liver with a normal fatty acid content in a diabetic patient who died of nephritis and pneumonia (case of Dr. F. D. W Lukens).

5. Wohlwill, F.: Klin. Wchnschr. **7**:344, 1928.

6. Dupérie, R., and Maupetit: Bull. et mém. Soc. méd. et chir. de Bordeaux **38**:317, 1935.

7. Brian, E. W.; Schechter, A. J., and Parsons, E. L.: Arch. Int. Med. **59**: 685, 1937.

8. Popper, H., and Wozasek, O.: Virchows Arch. f. path. Anat. **279**:819, 1931.

9. Kaufmann, E.: Spezielle pathologische Anatomie, Berlin, W. de Gruyter & Co., 1931.

Such observations are not confined to autopsy material, Stetson and Ohler [10] having described a clinical case.

Warren [1a] made the following statement:

> While it has been generally assumed that the increase in size and weight of the liver in diabetes is due to fat, this does not exclude other factors. Enlargement of the liver has been shown to be due to glycogen storage, to circulatory changes, amyloidosis, metastatic tumor as well as fat. Of much importance is the fluid content of the liver . . . large amounts of fat in the liver do not preclude the presence of normal or even large amounts of glycogen.

All of the physiologic processes involved in the deposition of glycogen are not entirely clear, but in the diabetic organism, in the presence of adequate dextrose glycogen is stored in the liver under the influence of insulin. This may have been the mechanism involved in the reported cases.

The pancreas was small or the islet tissue scanty in the 2 cases reported here and in several of the other cases cited,[11] as well as in the 2 cases reported by Moore [12] and Bowen and Beck.[13] In the latter

Pancreatic Weights in Adults with Diabetes

	Weight of Pancreas					
	To 50 Gm.	50-70 Gm.	70-90 Gm.	90-110 Gm.	110-130 Gm.	130 Gm. Up
Patients with diabetes.....	9	13	9	14	2	7
Nondiabetic patients.....	0	6	22	12	8	6

instances both patients were young diabetic subjects with convulsions (hypoglycemic?). In Brian, Schechter and Parsons' [7] case, however, the pancreas was normal.

This finding in the group of cases under discussion may have no special significance, for Warren [1a] found the pancreas weighing less than 50 Gm. in 74 of a series of 449 cases of diabetes (approximately 16 per cent). In a much smaller series of cases, studied in the department of pathology of the University of Chicago, in which the patients were adults, the pancreatic weights, contrasted with those of a control group, were as shown in the accompanying table.

It is interesting that the percentage of cases with the pancreas weighing less than 50 Gm. is 16 per cent, duplicating Warren's figure.

SUMMARY

Two cases in which glycogenic hepatomegaly was associated with insulin hypoglycemia in patients with diabetes are reported, and similar cases in the literature are reviewed.

10. Stetson, R. P., and Ohler, W. R.: New England J. Med. **217**:627, 1937.

11. Warren.[1a] Terplan and others.[2a] Dupérie and Maupetit.[6] Popper and Wozasek.[8]

12. Moore, R. A.: Am. J. Dis. Child. **52**:627, 1936.

13. Bowen, B. D., and Beck, G.: Ann. Int. Med. **6**:1412, 1933.

LIPOID HISTIOCYTOSIS

Report of a Case with Postmortem and Chemical Studies of the Spleen

ARCHIE H. BAGGENSTOSS, M.D.; EDWARD F. ROSENBERG, M.D., AND ARNOLD E. OSTERBERG, PH.D., ROCHESTER, MINN.

In recent years reports of a somewhat ill defined group of diseases characterized by a systemic proliferation of the reticuloendothelial system have appeared in the medical literature with increasing frequency. In most of the cases it has been impossible to establish the cause, and, owing to the varied clinical and pathologic pictures, it has been difficult to classify these diseases satisfactorily. Such attempts have been made, however, by Epstein,[1] Jaffé [2] and others. Sacks, in 1938, basing his classification on Epstein's work, listed the following four groups under the generic term "histiocytomatoses"; (1) diseases in which there is disturbance in the storage of lipoid, such as Gaucher's disease, Niemann-Pick disease and the Hand-Schüller-Christian syndrome; (2) infectious proliferative granuloma; (3) hyperplasia (reticuloendotheliosis, aleukemic reticulosis), and (4) dysplastic conditions, including so-called endothelioma. Perhaps the best defined and delimited group is the first. The diseases in this category have generally been considered as disturbances in lipoid metabolism or storage. In this group, the rather clearcut clinical and histologic pictures have been augmented by exact chemical analysis of the lipoids involved. Thus, Gaucher's disease has been designated as cerebroside lipoidosis; Niemann-Pick disease has been called phosphatide lipoidosis, and the Hand-Schüller-Christian syndrome and xanthomatosis have been termed cholesterol lipoidosis.

That the reticuloendothelial system is chiefly involved in these diseases is admitted by all, as the lipoids are in each instance accumulated within the cells of this system. Whether the disease rests on a primary dysfunction or hyperfunction of the cells of this system or whether the role of the reticuloendothelial system is passive or secondary to some other disturbance, such as one of lipoid metabolism, is in dispute and will be considered later. The relationship of lipoid histiocytosis (Sacks' group 1) to hyperplasia of the reticuloendothelial system (Sacks' group 3) is also not clear. It is possible that this relationship is more intimate than is generally suspected. Thus, it should be pointed out that in most of the reported cases of hyperplasia of the reticuloendothelial system (Sacks' group 3) the presence of large mononuclear cells in the organs affected is an almost constant observation. Explanations as to why the cells are large or as to what they contain are seldom given. Some authors have stated that the tissues involved are negative to stains for

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From the Sections on Pathologic Anatomy and Clinical Biochemistry of the Mayo Clinic.

1. Epstein, E.: Med. Klin. **21**:1501, 1925.

2. Jaffé, R. H.: The Reticulo-Endothelial System, in Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 2, p. 973.

various types of fat. As a rule, however, chemical studies have not been made, and the question as to whether or not these large cells contain lipoids cannot be settled by staining methods. If chemical studies of tissues were carried out in all cases of suspected reticuloendotheliosis, it might help a great deal in clearing up the relationship of these diseases and lead to clearer conceptions as to their genesis.

REPORT OF CASE

The patient was a young woman, aged 20 years, who was of English descent. When she registered at the Mayo Clinic, Oct. 23, 1938, because of vomiting which had been present for the past seven weeks, she was admitted directly to the hospital. No relevant information could be elicited regarding the family. Nine siblings were well. Her own past history was likewise irrelevant; she had had pneumonia at the age of 10 years.

Seven weeks before she came to the clinic she began to have attacks of vomiting. At times the attacks were preceded or accompanied by sensations of vertigo. Vomiting increased in intensity. Between Sept. 15 and 23, 1938, she vomited many times daily and also had some fever. No cause of this illness could be determined by her family physician, but, at the insistence of her parents, an exploratory laparotomy was performed on September 23. No lesions which might explain the vomiting were encountered; the appendix, which was the site of slight chronic inflammation, was removed. After the operation the vomiting persisted; it occurred whenever the patient ate, and it frequently was precipitated by quick movements. The patient had a daily fever which continued until she came to the clinic. At times her temperature reached 102 F. For two weeks before the patient came to the clinic slight epistaxis occurred occasionally. On the day of her arrival she noted that her urine was bloody.

When the patient was examined, she was very pale and obviously acutely ill. Her lips were dry and fissured, and numerous petechial hemorrhages were noted about the soft palate, finger tips and left conjunctival sac. The temperature was 102 F. on admission. The pulse rate varied from 100 to 148 per minute during her stay in the hospital. The breasts were not abnormal. The heart was enlarged to the left. There was evidence of congestion at the bases of both lungs. These findings were corroborated by roentgenologic examination of the thorax. There was an enlarged spleen. There was no evidence of ascites, and the pelvis and rectum revealed nothing of importance. The systolic blood pressure was 110 mm. of mercury, and the diastolic pressure was 70 mm.

The specific gravity of the urine was 1.014. There was albuminuria of grade 1, and 12 pus cells appeared in each field of the microscope as viewed under the high power objective. The value for hemoglobin was 6.3 Gm. in 100 cc. of blood. There were 2,090,000 erythrocytes and 11,500 leukocytes per cubic millimeter of blood. The sedimentation rate of the erythrocytes was 120 mm. in one hour. A blood smear disclosed toxic changes, grade 2 to 3, in the polymorphonuclear neutrophils, a shift to the left and immature forms of leukocytes extending as far back as the so-called stem cells. The anemia appeared to be of a secondary type. The appearance of the smear, including the toxic changes in the polymorphonuclear neutrophils and the presence of immature leukocytes, was interpreted as probable evidence of a leukemoid reaction, but the possibility that the patient was suffering from chronic myelogenous leukemia could not be definitely excluded.

The patient failed rapidly. The temperature remained elevated continuously and varied between 100.4 and 103.4 F. until her death, which occurred October 25, two days after her registration.

The body was promptly embalmed, and necropsy was made three hours after death. The body was 163 cm. in length and was estimated to weigh 130 pounds (59 Kg.). There was no evidence of edema, and the incision in the lower right quadrant of the abdomen had healed. Numerous petechial hemorrhages were seen in the skin over the anterior portion of the thorax, arms and legs, and a somewhat larger effusion of blood was found in the subcutaneous tissues in the midline of the abdomen. The omentum was adherent to the abdominal incision and to the cecum at the site of the recent appendectomy. There was no excess of fluid in the abdominal cavity. Approximately 50 cc. of clear straw-colored fluid was found in each thoracic cavity. The lungs were not adherent. Spread irregularly about the pleural surfaces of both lungs were numerous small patches of thickening, which were whitish in appearance and measured 2 to 3 mm. in diameter. These had the appearance of patches of leukemic infiltration. The lungs showed slight edema of the lower lobes, but otherwise they were essentially normal in appearance. No evidence of tuberculosis, healed or active, was encountered. The pericardial sac contained approximately 100 cc. of clear yellow fluid. The heart weighed 292 Gm. Several minute petechial hemorrhages were noted beneath the pericardium about the anterior surface of the left ventricle. Yellowish subendocardial streaking in the wall of the right ventricle gave the impression of lipoid degeneration of the myocardium. Otherwise the heart was not abnormal.

The spleen weighed 704 Gm. Its surface was grayish white and was speckled with white nodules of increased density. These varied in size; some were as large as 4 mm. in diameter. The capsule itself was smooth and glistening but otherwise was not thickened. The small nodules rose slightly above the surface of the capsule. Multiple similar whitish nodules were distributed widely about the cut surface and had replaced a considerable quantity of the splenic pulp (fig. 1). The remaining splenic substance was light red.

The liver weighed 2,570 Gm. Its surface was studded irregularly with occasional nodules, which were as large as 3 or 4 mm. in diameter and were similar in appearance to those noted in the spleen. The gross appearance of the sectioned surface was not abnormal.

The marrow in the vertebrae of the thoracic and lumbar segments of the spinal column contained numerous grayish yellow irregular softened regions which varied from a few millimeters to 3 cm. in diameter.

The lymph nodes about the hili of the lungs and about the pancreas, mesentery and aorta were not enlarged. The entire gastrointestinal tract and the pancreas, kidneys, adrenal glands, ureters, bladder, uterus, thymus and thyroid gland were normal. There were multiple follicular cysts in both ovaries. Atherosclerosis of the aorta, grade 1, was present. The brain weighed 1,147 Gm. and appeared normal throughout. The spinal cord was normal in appearance. The pituitary body likewise appeared normal.

Histologic Observations.—The most significant histologic changes were in the spleen, bone marrow, liver, lungs and lymph nodes.

(*a*) Spleen. Paraffin sections which were stained with hematoxylin and eosin revealed almost complete destruction of the normal architecture by a massive proliferation of large, faintly staining cells, which appeared as a syncytium in many regions (fig. 2*A*). These cells were present apparently throughout the spleen, and in the grossly visible grayish white nodules they were the only elements to be seen. In regions in which the cellular hyperplasia was less advanced the proliferating cells appeared to take their origin from the cytoplasmic reticulum rather than from the cells lining the sinuses. Occasional large reticular cells were seen lying in the sinuses, however.

The abundant cytoplasm of the hyperplastic cells was faintly eosinophilic or neutrophilic and contained numerous tiny vacuoles which failed to take the stain (fig. 2*B*). The boundaries of these cells were not distinct, and in many regions in which the cells were closely packed no definite borders could be seen. In some cells the cytoplasm extended out into long strands or sail-like projections, while other cells, which were more closely packed, were polyhedral or oval. In general, the cells were large and varied from 10 to 18.5 microns in diameter.

The nuclei, although rather large, appeared small in relation to the cytoplasm. They generally measured about 7 microns in diameter. Most of them were oval or kidney shaped. The chromatin was arranged as a network; there were condensations of it at various points which gave the nucleus a granular appearance. The Pappenheim and Dominici [2a] methods of staining revealed the delicate nature

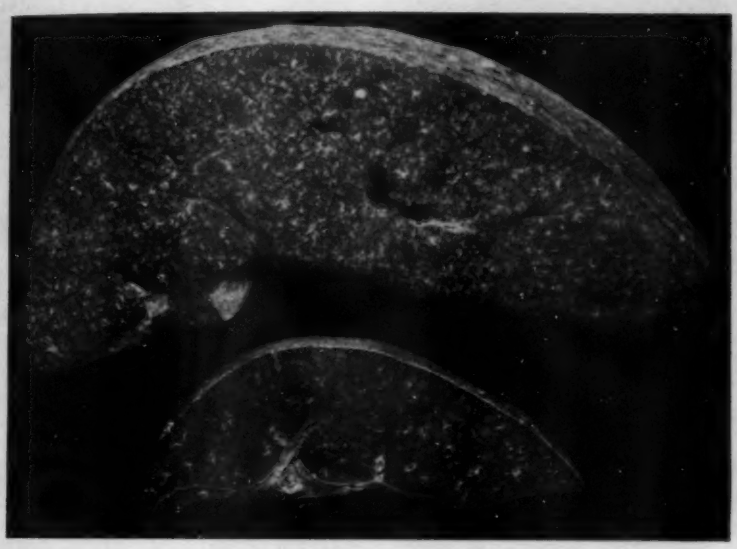

Fig. 1.—At the top is seen a cross section of the spleen, showing nodules and hypertrophy; at the bottom, a cross section of a normal spleen.

of the chromatin network and emphasized the vacuolation of the cytoplasm. Multinucleated cells were numerous, and some of them appeared similar to the Sternberg-Reed cells of Hodgkin's disease (fig. 2*C*). Other characteristic features of Hodgkin's disease, such as eosinophils, fibrosis and necrosis, were absent. The homogeneity of the cellular elements tended to eliminate the presence of Hodgkin's disease.

Some of the proliferated cells appeared to have phagocytic ability, as they contained erythrocytes and cellular debris. In sections which were stained for iron many of the cells were found to contain hemosiderin in varying amounts. In the regions in which proliferation appeared most rapid there was very slight or no evidence of phagocytosis.

2a. Dominici, M.: Compt. rend. Soc. de biol. **54**:221, 1902.

Sections impregnated with silver by the methods of de Galantha [3] and Bielschowsky revealed a network of reticulum fibers in intimate relationship with these cells. In regions in which proliferation of the cells led to the formation of distinct nodules these fibers were few in number and very fine. Sections stained by Mallory's phosphotungstic acid hematoxylin stain likewise revealed these reticulum fibers.

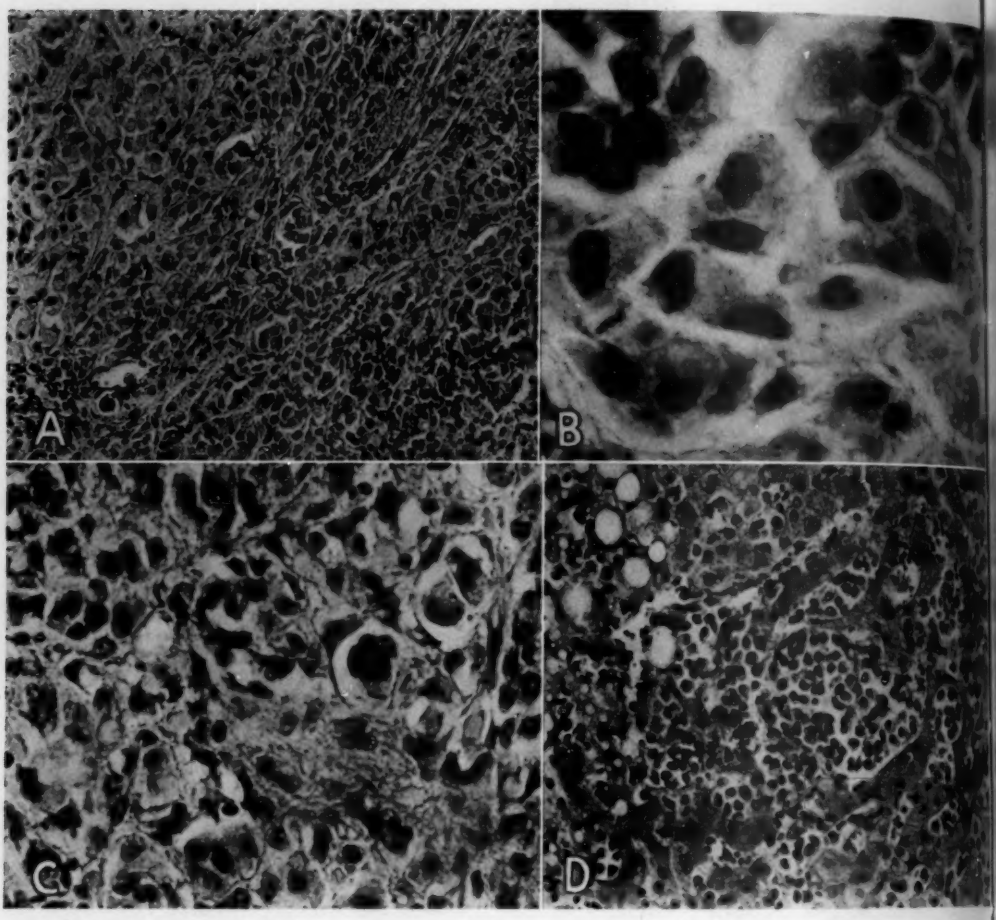

Fig. 2.—*A*, section obtained from the edge of a nodule in the spleen; hematoxylin and eosin; × 180. *B*, same section as shown in *A*; × 1,000. *C*, giant cells in the spleen; hematoxylin and eosin; × 450. *D*, proliferation of cells in the liver; stained with hematoxylin and eosin; × 200.

Frozen sections stained with sudan III and scarlet red did not reveal any fat. The results obtained with the Lorrain Smith–Dietrich stain were inconclusive as to the presence of lipoids, but sections stained by Ciaccio's method revealed small

3. de Galantha, E.: Personal communication to the authors.

yellowish orange droplets in the cytoplasm of some of the cells. These are supposedly indicative of the presence of phosphatides. The results obtained with Weigert's myelin sheath stain were negative.

(*b*) Liver. Sections of this organ were interesting, as the proliferating cells, although identical in appearance and staining reactions with those of the spleen, appeared to arise from the Kupffer cells lining the sinusoids (fig. 2 *D*). In sections taken from the edge of the nodules which were grossly visible there were definite transitions between the Kupffer cells and the hyperplastic elements. Some of these cells were still attached to the walls of the sinusoids, while others were lying free in the lumen. In some regions the proliferation was so advanced that the sinusoids were distended by solid groups of cells. In other regions in which the cells retained their connection with the walls of the sinuses the hyperplasia had resulted in the formation of pseudoacini.

(*c*) Bone marrow. Sections were obtained from the sternum, vertebrae and the petrous portion of the temporal bone. The cells were similar in appearance and staining reactions to those in the spleen and liver (fig. 3 *A*). The staining methods of Pappenheim and Dominici demonstrated vacuolated cytoplasm and a faintly staining delicate network of chromatin in the nucleus. The cells appeared to arise from the cells lining the sinusoids and formed solid groups and pseudoacini like those seen in the liver. Multinucleated cells and mitotic figures were numerous. In some regions the cellular hyperplasia had completely replaced the normal marrow, and there was resorption of the trabeculae. There was no evidence of anything resembling normal hemopoiesis in these proliferating elements. In one of the vertebrae the process had broken through the cortical bone and had lifted up the periosteum.

Touch preparations of sternal and vertebral marrow were also made and stained with Wright's stain and Giemsa's stain. These preparations contained numerous large cells with faintly staining vacuolated cytoplasm and nuclei which had a very delicate, finely stippled chromatin network. The majority of the cells had the typical structure of reticuloendothelial cells.

(*d*) Lymph nodes. The only lymph nodes which appeared to share in the hyperplasia were those at the hilus of the lung. In these the proliferation had resulted in the formation of nodules, which were not sharply demarcated but seemed to fuse gradually with the surrounding lymphatic tissue. In the region of this nodular hyperplasia the normal architecture of the node had completely disappeared. Some of the nodes showed profuse proliferation and mobilization of the littoral cells lining the lymph sinuses, similar to that seen in various inflammatory processes (fig. 3 *B*). In some regions the newly proliferated cells, both the reticulum and the lining cells, contained large amounts of carbon pigment (fig. 3 *C*). The staining reactions of the cells were identical with those of the cells of the spleen.

(*e*) Lungs. Sections through the macroscopically visible nodules in the lungs revealed regions of cellular hyperplasia identical with those already described (fig. 3 *D*). In addition, there were small foci of cellular proliferation about the small blood vessels. In these foci the cells appeared to arise from the adventitia of the vessels.

In many regions the alveolar walls appeared thickened as a result of an increased number of large mononuclear cells, which possessed vacuolated cytoplasm and appeared similar to those already described. Considerable numbers of these cells were also free in the alveoli and contained phagocytosed erythrocytes and hemosiderin. In other portions of the lung there was evidence of edema, and in a few

foci there was a slight fibrinous exudate, which contained very few cellular elements, chiefly mononuclear cells.

(*f*) Breast and ovary. In routine sections of the left breast and right ovary there were a few minute foci of cellular proliferation which were identical with those already described. These cells appeared to arise from the connective tissue.

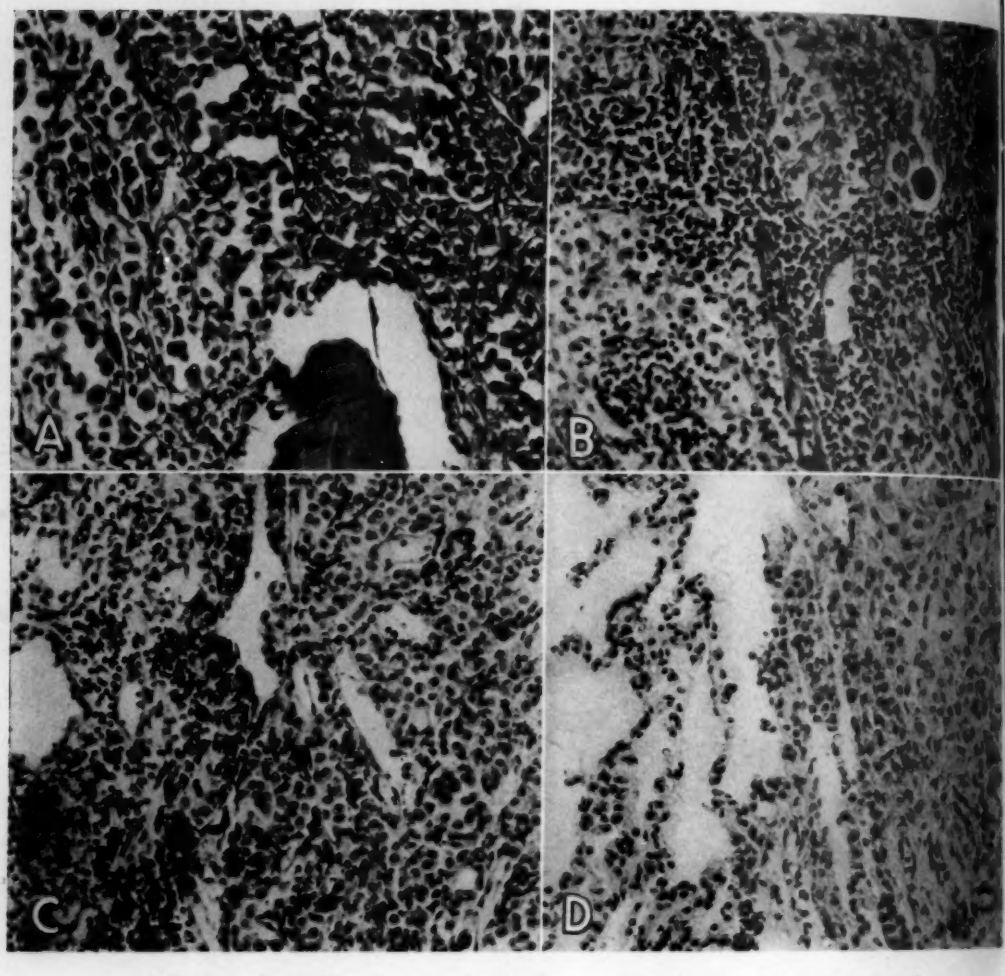

Fig. 3.—*A*, proliferation of cells in sinuses of the bone marrow; hematoxylin and eosin; × 200. *B*, proliferation and mobilization of littoral cells in sinuses of a hilar lymph node; hematoxylin and eosin; × 200. *C*, proliferating cells in a lymph node, some of which reveal phagocytosis of carbon pigment; hematoxylin and eosin; × 200. *D*, section of a pulmonary nodule; hematoxylin and eosin; × 200.

(*g*) Heart. Frozen sections of the right ventricle of the heart stained with sudan IV revealed numerous fine lipoid granules in the muscle fibers.

(*h*) Other organs. Sections of the kidneys, adrenal glands, thymus, uterus, pancreas, thyroid gland and bladder were normal.

In view of the fact that the histologic appearance of the organs was in many respects similar to that of the tissues in Gaucher's disease, the spleen was analyzed for lipoids according to the method of Lieb.[4] At the same time an analysis for lipoids was made on a spleen which had been removed at operation in a case of Gaucher's disease. In table 1 (prepared from the data of Epstein and Lorenz [5]) are presented chemical data which distinguish the lipid diseases. In this table it may be seen that Niemann-Pick disease is associated with a marked increase in the phosphatide and lecithin, whereas Gaucher's disease is distinguished by a very large content of cerebroside kerasin. In the Hand-Schüller-Christian syndrome, which is one of the xanthomatoses, cholesterol and cholesterol esters are stored in the diseased tissues

TABLE 1.—*Concentration of Kerasin and Lipoids in a Normal Spleen, in a Spleen Involved in Niemann-Pick Disease, in a Spleen Involved in Gaucher's Disease and in a Mass Removed from Dura Mater Involved in the Hand-Schüller-Christian Syndrome*

	Concentration, Expressed as Percentage of Weight of Dried Organ or Tissue			
	Normal Spleen	Spleen Involved in Niemann-Pick Disease Phosphatide Lipoidosis	Spleen Involved in Gaucher's Disease Cerebroside Lipoidosis	Mass from Dura Mater Involved in Hand-Christian-Schüller Syndrome Cholesterol Lipoidosis
Kerasin	0	0	10	0
Cholesterol	0.62	0.73	Trace	3.2
Cholesterol esters	0.287	0.68	Trace	15.3
Total cholesterol	0.9	1.41	Trace	18.58
Lecithin	1.66	13.17	1.46	1.60
Neutral fat	4.183	12.82	Trace	14.4
Total lipoids *	4.6	12.91	Trace	31.58

* The figures for this were obtained as follows: 10 per cent was subtracted from the value for neutral fat. This 10 per cent represents the approximate weight of glycerol in the fat molecule. To the resulting figure was added the value for total cholesterol. Thus, for the spleen of the patient with Niemann-Pick disease observed by Epstein and Lorenz the calculations were as follows: spleen 12.82 — 1.3 = 11.5 (total fatty acids); 11.5 + 1.41 = 12.91 (total lipoids). The values for total lipoids obtained in this manner may be compared with the values for total lipoids in table 2, and indicate the total combined weight of fatty acids and cholesterol with cholesterol esters.

in large quantities. In table 2 are presented the results of an analysis of the two spleens. Both show an increase in total lipoids and cholesterol (table 1). The spleen in the case of Gaucher's disease contained a large quantity of the cerebroside kerasin whereas only a small amount of this was found in the spleen in the present case. Lecithin [6] was found in significant amounts in the spleen in the present case but not in the spleen in the case of Gaucher's disease.

4. Lieb, H.: Ztschr. f. physiol. Chem. **140**:305, 1924.

5. Epstein, E., and Lorenz, K.: Ztschr. f. physiol. Chem. **192**:145, 1930.

6. Klenk (Baumann, T.: Klenk, E., and Scheidegger, S.: Ergebn. d. allg. Path. u. path. Anat. **30**:183, 1936) recently claimed that the phospholipid which is found in the cells in Niemann-Pick disease is not composed entirely of lecithin but consists largely of the diaminophosphatide sphingomyelin. Baumann (Baumann, T.: Klin. Wchnschr. **14**:1743, 1935) and Tropp (Tropp, C.: ibid. **15**:562, 1936) have confirmed this. In order to simplify our study, the entire ether-soluble phospholipid fraction of the spleen is reported as lecithin.

COMMENT

Consideration of the data in tables 1 and 2 suggests that the disease which we have reported could not be classified definitely in any of the groups of disorders of the storage of lipoids. The presence of considerable quantities of lecithin and of a large amount of total lipoids suggests Niemann-Pick disease, but this is probably excluded by the clinical data, for Niemann-Pick disease has been noted for its tendency to affect infants and to cause death during the first few years of life. From a chemical standpoint, the dysfunction of fat metabolism in our patient was probably of a type intermediate between that of Gaucher's disease and that of Niemann-Pick disease.

In recent years a number of workers have indicated that from a chemical standpoint an overlapping of these three diseases occurs frequently. Thus Sobotka, Epstein and Lichtenstein [7] pointed out that in many of the reported cases of Niemann-Pick disease an examination of tissues has disclosed not only an accumulation of lecithin but also a considerable increase in the amount of cholesterol (3.1 to 6.6 times the normal amount). Clément [8] agreed with them in this opinion and said that as observations multiply one sees that the original classifications do not take care of all cases, as delimitations are artificial and many clinical phenomena overlap. Hamperl [9] also reported an instance in which overlapping of the metabolic fault was definitely present. In a case of Gaucher's disease he found a considerable amount of lecithin in the spleen (5.98 per cent), nearly as much as Bloom and Kern [10] found in a case of Niemann-Pick disease. Pick [11] also held this view; in the Dunham lecture, which was delivered at Harvard University in 1932, he stated that a number of deviations which exist do not fit into rigid schemes. Our data suggest that the disease condition reported belongs in this group of deviations mentioned by Pick.

This condition presented a difficult diagnostic problem to both the clinician and the pathologist. The symptoms and signs suggested an infection, and subacute bacterial endocarditis was considered, but this diagnosis could not be substantiated. Acute leukemia was suggested, but this diagnosis was not definitely supported by the blood smears, which pointed rather to a leukemoid reaction or to chronic myelogenous leukemia. The rapid downhill course precluded further laboratory studies, and the patient died before a definite diagnosis could be made.

At necropsy the disease likewise presented difficulties in diagnosis. No infectious process could be demonstrated. The enlarged spleen and the nodules in the liver, lungs and bone marrow, considered together with the findings on the blood during life, suggested chronic myelogenous leukemia. Frozen sections of these tissues, however, did not support this diagnosis but pointed rather to lipoid histiocytosis, possibly Gaucher's disease. For this reason the chemical studies were carried out, with results as already noted (table 2).

7. Sobotka, H.; Epstein, E. Z., and Lichtenstein, L.: Arch. Path. **10**:677, 1930.
8. Clément, R.: Rev. de méd., Paris **55**:219, 1938.
9. Hamperl, H.: Virchows Arch. f. path. Anat. **271**:147, 1929.
10. Bloom, W., and Kern, R.: Arch. Int. Med. **39**:456, 1927.
11. Pick, L.: Am. J. M. Sc. **185**:601, 1933.

Further histologic study did not support the initial impression that we were dealing with a typical form of either Gaucher's disease or Niemann-Pick disease. The lipoid containing cells were not as large as those described for these diseases, while the nuclei were larger than those usually described. The vacuolated cytoplasm of some of the cells, however, resembled that found in Niemann-Pick disease. Ciaccio's method of staining revealed phospholipoid in occasional cells. This also suggested Niemann-Pick disease. None of the cells possessed the fibrillated cytoplasm characteristic of Gaucher's disease. The presence of numerous mitotic figures indicated more active hyperplasia than is usually seen in lipoid histiocytosis. The microscopic appearance of the lesions was similar in many respects to that of a neoplastic process and the possibility of retothelial sarcomatosis as outlined by Roulet [12] and Benecke [13] was considered.

In the bone marrow, especially, the behavior of the hyperplastic process, including the resorption of bone trabeculae and actual erosion

TABLE 2.—*Concentration of Kerasin and Lipoids in a Spleen Involved in Gaucher's Disease and in a Spleen Involved in Atypical Lipoid Histiocytosis*

	Concentration, Expressed as Percentage of Weight of Dried Organ	
	Spleen Involved in Gaucher's Disease	Spleen Involved in Atypical Lipoid Histiocytosis*
Kerasin	11.0	0.37
Cholesterol	2.42	1.97
Lecithin	Trace	2.20
Total fatty acids	6.14	9.30
Total lipoids	8.57	11.10

* Unfortunately the spleen had already been fixed in modified Kaiserling's solution, and consequently the values obtained for lecithin were probably lower than the actual amounts present at the time of death. The content of kerasin, cholesterol and neutral fat in tissues is not altered by exposure to fixing solutions.

of the cortex, was that of a neoplasm. The lesions in the liver and spleen, however, resembled hyperplasia of preexisting Kupffer cells and reticulum cells rather than a new growth. Multiple myeloma was also considered, but the diagnosis could not be substantiated. Clinically and histologically there were many similarities between this case and the cases of acute reticuloendotheliosis reported by Uehlinger,[14] Ugriumow,[15] Sacks [16] and others. Jaffé summarized the available information from such cases, and it is not necessary to review the data here. Suffice it to say that the insidious onset, weakness, fever, petechiae, splenomegaly, inconclusive hematologic picture and hyperplasia of the cells of the reticuloendothelial system in this case are all features which have been described in many reports of cases of acute reticuloendotheliosis which he

12. Roulet, F.: Virchows Arch. f. path. Anat. **286**:702, 1932.

13. Benecke, E.: Virchows Arch. f. path. Anat. **286**:693, 1932.

14. Uehlinger, E.: Beitr. z. path. Anat. u. z. allg. Path. **83**:719, 1930.

15. Ugriumow, B.: Zentralbl. f. allg. Path. u. path. Anat. **42**:103, 1928.

16. Sacks, M. S.: Arch. Path. **26**:676, 1938.

has reviewed. If determinations of the concentration of lipoid in the spleen had not been carried out in this case, the disease would probably have been considered as acute reticuloendotheliosis.

Some of the clinical symptoms of the patient can be explained by the observations at necropsy. The cause of the persistent vomiting is not certain. Because of the involvement of the marrow of the petrous portion of the temporal bone it was thought that there might be interference with the vestibular apparatus of the internal ear. This deduction could not be substantiated, however, although numerous sections were taken through the internal ear. The anemia, petechiae and epistaxis were probably the result of the reduction of functioning marrow by the hyperplastic process.

The point that should be emphasized here is that this condition clinically and histologically fell under the heading of hyperplasia of the reticuloendothelial system as classified by Sacks [16] (group c) but that when chemical determinations were made the unusual quantities of lipoid constituents of the spleen placed the disease in the group of lipoid histiocytosis. To us, this suggested the possibility that if lipoids were determined in all cases of reticuloendotheliosis a great many more disease conditions would be placed in this group rather than in that of simple hyperplasia. It also suggested the possibility that the relationship between these two groups may be much more intimate and fundamental than was believed.

The widely accepted view of Epstein and Pick [17] is that the diseases known as lipoid histiocytosis (Gaucher's disease, Niemann-Pick disease, and the Hand-Schüller-Christian syndrome) are disturbances of the storage of lipoid. Thus Pick advanced the theory that the role played by the reticular cells is of secondary importance and that the primary condition is a disturbance of metabolism. In other words, the substance which appears in the cells is not the product of abnormal activity of these cells but arises extracellularly and is merely stored by these cells. Recent investigation suggests, however, that this view may be in error inasmuch as there is now some evidence of abnormal metabolism in these cells. It is known, for instance, that the cerebroside kerasin is a galactoside derived from the ceramid lignoceryl-sphingosin by combination with galactose. Fraenkel and Bielschowsky [18] demonstrated lignoceryl-sphingosin in the normal liver, and, according to Thannhauser,[19] this substance occurs only in organs that are rich in reticular cells. Tropp [20] said that since lignoceryl-sphingosin is a normal product of the reticuloendothelial cells, it may be that in these cells the fermentative synthesis of the galactoside kerasin takes place. Tropp assumes that normally the kerasin which is formed by the reticuloendothelial cells is carried to the nerve tissues to be used for the building up of myelin. In Gaucher's disease, then, the kerasin accumulates in the organs of the reticuloendothelial system either because of a disturbance in the mobilization and transportation of the finished product to the brain, spinal cord and

17. Pick, L.: Ergebn. d. inn. Med. u. Kinderh. **29**:519, 1926.

18. Fraenkel, E., and Bielschowsky, F., cited by Baumann, Klenk and Scheidegger in footnote 6.

19. Thannhauser, S. J.: Klin. Wchnschr. **11**:1693, 1932.

20. Tropp, C.: Klin. Wchnschr. **15**:562, 1936.

myelinated nerves or because of excessive formation of kerasin by the reticuloendothelial cells. Likewise in Niemann-Pick disease, Dienst and Hamperl [21] and Tropp assume that the dysfunction of the reticuloendothelial system is primary and the lipoid changes are secondary.

Recent work indicates also a close basic relationship between Gaucher's disease and Niemann-Pick disease. Klenk,[20] for example, was able to show that the organs in Niemann-Pick disease contained large amounts of the diaminophosphatide sphingomyelin. In the liver it formed 23.8 per cent and in the spleen 24.7 per cent of the dried substance, whereas the glycerophosphatides (such as lecithin) were not increased. Baumann and Tropp [20] reported similar findings. It was pointed out by these men that sphingomyelin and kerasin have as a common base lignoceryl-sphingosin. In Gaucher's disease this base combines with galactose to form kerasin, while in Niemann-Pick disease it combines with choline to form the diaminophosphatide sphingomyelin.

According to this hypothesis, the amount and character of the lipoids in the affected organs depend on the synthetic activity of the reticuloendothelial cells. Depending on the direction and degree of this activity, different types and amounts of lipoids might be produced. Such a hypothesis would explain conditions such as that encountered in our case which fits neither group of diseases satisfactorily.

There appears to be a need for thorough chemical studies of the involved tissues not only in cases of lipoid histiocytosis but in all cases of reticuloendotheliosis, whether or not disturbances in lipoid metabolism are suspected. We feel that such studies may lead to more complete information regarding the nature of reticuloendotheliosis and its relation to lipoid metabolism.

SUMMARY

The atypical lipoid histiocytosis which we have reported does not fit into the present classification of such diseases. Other reports which have appeared indicate that in these diseases there is considerable overlapping as regards the character and amount of the lipoid constituents of the organs involved. Recent investigation also tends to show that dysfunction or unexplained hyperplasia of the reticuloendothelial system may be primary and play an active rather than a passive part in the genesis of these diseases. It is believed that if chemical determinations were carried out in all cases of so-called primary hyperplasia of the reticuloendothelial system as well as in cases of typical lipoid histiocytosis a better understanding of these disorders might result.

21. Dienst, G., and Hamperl, H., cited by Jaffé.[2]

Laboratory Methods and Technical Notes

A COMPARISON OF STARCH PASTE AND ALBUMIN MIXTURE AS AGENTS FOR THE ROUTINE MOUNTING OF PARAFFIN SECTIONS

ANNA MARY MCDOWELL, M.A., AND GEORGE A. VASSOS JR., M.D., NEW YORK

The albumin-glycerin mixture [1] for affixing paraffin sections to slides has been used routinely in many pathologic laboratories and has undoubtedly been one of the best preparations for this purpose. However, since Spoerri [2] suggested the use of a starch paste for mounting sections of nerve tissue, the use of such a starch suspension has been tried in this laboratory for routine affixation of paraffin sections of all surgical pathologic specimens. It is because of the advantages afforded by the use of this suspension that we feel a comparison of the methods is warranted.

The albumin-glycerin mixture [1] is made up by taking equal parts of egg white and glycerin, filtering, and adding a small crystal of thymol as a preservative. The starch paste tried is essentially the same as the one described by Spoerri.[2] One gram of powdered starch is added to 10 cc. of cold water and thoroughly mixed; the solution is then poured into 20 cc. of boiling water, 2 drops of dilute hydrochloric acid is added, and the suspension is boiled for five minutes while being constantly stirred to free the opalescent suspension from lumps of starch. A small crystal of thymol should be added after the paste has cooled.

The clean glass slides are prepared by coating one surface with a thin film of the mixture to be used. Drying of the affixative on the slides does not seem to be a disadvantage as long as no dust is allowed to collect on them.

Paraffin sections are then flattened out on the surface of warm water and floated onto the slides already smeared with the adhesive to be used. Sections thus mounted on slides are ready for drying prior to their deparaffinization and staining.

Several methods of drying have been tried, but it is felt that the following are the most effective and time saving in the particular staining technic concerned, although it is recognized that longer drying periods at lower temperatures may be used successfully. For a modified Masson trichrome stain,[3] the ordinary hematoxylin and eosin method, a modified Giemsa procedure [4] and the Foot silver impregnation for brain tissue,[5] the following drying method is considered effective and adequate:

Rest one end of each slide on the edge of a tray so that the slides will be in a slanting position. The tray with the slides is then put on an electric hot plate

From the Department of Surgical Pathology of the New York Hospital and Cornell University Medical College.

1. Meyer, P.: Mitt. zool. Station, Neapel **4**:521, 1883.
2. Spoerri, R.: Science **90**:260, 1939.
3. Goldner, J.: Am. J. Path. **14**:237, 1938.
4. Wolbach, S. B.: J. M. Research **41**:1, 1919-1920.
5. Foot, N. C.: Am. J. Path. **14**:245, 1938.

and left there for about three minutes after the paraffin has begun to melt. The sections attached to the slides in such a manner are then put into an oven and kept at 56 to 58 C. for one hour or longer. The sections are now ready for deparaffinization before staining.

A comparison of the adhesive qualities of the albumin and the starch affixatives shows that there is no advantage of one over the other in the staining procedures noted.

With the Ziehl-Neelsen carbol fuchsin method,[6] the three minutes on the hot plate and one hour in the drying oven at 56 to 58 C. are found to be effective and adequate when the albumin preparation is used, but longer drying is necessary when starch is used as an adhesive. After the sections are floated onto the slides filmed with the starch paste, the slides are put in an oven and kept at 38 to 40 C. for about twenty-four hours. The sections are then found to adhere well while being subjected to this staining technic.

Table Illustrating Temperature and Time Necessary for Drying Sections to Insure Good Adhesion of Paraffin Sections to Slides During Use of Staining Technics

Drying Method	Affixative	Modified Masson Stain[2]	Hematoxylin-Eosin	Modified Giemsa[4]	Foot Silver for Brain[5]	Ziehl-Neelsen[6]	Foot Silver for Reticulum[7]
On hot plate for 3 min. and in drying oven at 56-58 C. for 1 hr.	Albumin	A	A	A	A	A	I
	Starch	A	A	A	A	I	I
In drying oven at 38-40 C. for 24 hr.	Albumin	A	A	A	A	A	I
	Starch	A	A	A	A	A	I
In drying oven at 56-58 C. for 48 hr. or at 38-40 C. for 5 days	Albumin	U	U	U	U	U	A
	Starch	U	U	U	U	U	A

A = adequate; I = inadequate; U = unnecessary.

With the Foot method of silver impregnation for reticulum,[7] it is found that the albumin and the starch paste are equally effective as far as their adhesive qualities are concerned. However, a longer drying time has been found necessary for either affixative used. After sections have been floated onto the prepared slides and incubated at 56 to 58 C. for forty-eight hours, they adhere well. This is important as the chief complaint of technicians using this impregnation has always been directed against the detachment of sections from the glass slides.

The starch paste has no such affinity for any of the dyes in the various staining technics used as is occasionally seen with the albumin mixture, and therefore gives a definitely clearer background. This is especially true when the sections are impregnated with silver; the egg albumin tends to combine with the silver while the starch does not.

The staining properties of the various tissues are the same whether starch or albumin affixatives are used, but the most notable feature resulting in the starch-filmed slides is the clear background.

6. Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938, p. 276.

7. Foot, N. C., and Menard, M. C.: Arch. Path. **4**:211, 1927.

The fixation of tissues in formaldehyde-alcohol solution, Zenker's fluid or Bouin's fluid makes no variation in the adhesiveness with either paste used.

Other advantages of the starch over the albumin are its easy preparation, simplicity and cleanliness, which render it most suitable for routine affixing of paraffin sections to slides.

SUMMARY

It has been shown that a starch paste has some advantages over the albumin-glycerin mixture for affixing paraffin sections to slides in the course of several staining technics.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, Etc.—Max Askanazy, professor of pathologic anatomy at the University of Geneva, Switzerland, has reached the age limit and retired. His pupil Erwin Rutishauser was chosen as his successor.

Perrin Long has been appointed head of a new department, that of preventive medicine, in the school of medicine of Johns Hopkins University, Baltimore.

Fritz Strassmann, professor of forensic medicine at the University of Berlin from 1894 to 1930, when he retired, died Jan. 30, 1940, in his eighty-second year.

Bruno Galli-Valerio, professor of hygiene and bacteriology at the University of Lausanne, Switzerland, has retired after fifty years of teaching. Paul Hauduroy, director of the health department of Colombes, near Paris, has been appointed as his successor.

Alexander Besredka, of the Pasteur Institute in Paris, died Feb. 28, 1940, at the age of 70.

Medicolegal Conference.—February 23, a medicolegal conference was held in Chicago under the auspices of the Committee on Local Medicolegal Problems of the Institute of Medicine, Oscar T. Schultz, chairman. In the evening Alan R. Moritz, professor of legal medicine, Harvard Medical School, delivered an address on "Medical Science and the Administration of Justice."

Research in Problems of Sex.—Applications to the Committee for Research in Problems of Sex, of the National Research Council, for financial aid in support of the study of fundamental problems of sex and reproduction during the year beginning July 1 should be received before April 1. They may be addressed to Dr. Robert M. Yerkes, Yale School of Medicine, New Haven, Conn. Preference will be given to proposals for investigation of neurologic, psychobiologic and behavior problems.

Leon Bernard Prize.—The first award of the Leon Bernard Prize of the Health Committee of the League of Nations has been made to Wilbur A. Sawyer, director of the International Health Division of the Rockefeller Foundation, for his work on yellow fever and in the general field of preventive medicine.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Cytological Changes Induced in the Hypophysis by Pituitary Extract. A. E. Severinghaus and K. W. Thompson, Am. J. Path. **15**:391, 1939.

The hypophyses studied were from (*a*) 2 dogs given daily injections of 25 cc. of an extract of sheep pituitary for one hundred and twenty and two hundred and ten days, respectively; (*b*) 2 dogs treated subcutaneously for thirty-two and thirty days, respectively, with 10 cc. of "antihormone" serum from a dog that was treated daily for three years with an extract of sheep pituitary, and (*c*) 2 immature, 4 month old female sheep given daily injections of 25 cc. of an extract of sheep pituitary for six months. The cytologic changes in the first pair of animals were noted mainly in the basophilic cells. The granules clumped to aggregates of varying sizes, and vacuoles appeared in the cytoplasm, some colorless, some filled with a clear pale blue substance and some filled with a deeply basophilic amorphous material. The vacuoles tended to expand and to occupy most of the granular cytoplasm. In some cells these changes were identical with those observed in the hypophysis of the castrated rat or monkey; in other cells they resembled closely the changes seen in the pituitary gland of the thyroidectomized rat; still other cells showed the hyaline-like cytoplasmic changes that were described by Crooke in the Cushing syndrome of pituitary basophilism. The hypophyses of the second pair of animals presented a decrease in the number of chromophobe cells and tinctorial and structural changes in the basophilic and in the acidophilic cells, both types of cells occurring in increased numbers. Another change was marked hyperemia and edema. In the hypophyses of the 2 immature female sheep the most notable feature was an almost universal degranulation of the basophilic cells. Changes were also noted in the size and staining qualities of the acidophilic cells. A hypothesis is offered to explain the observed changes. The injected pituitary extract activates the endocrine glands related to the anterior lobe of the pituitary, namely, the gonads, the thyroid and probably the adrenals, which activation stimulates in turn the anterior lobe of the hypophysis to increased secretion. The injected heterologous pituitary extract stimulates the production of "antihormones," which neutralize at first the injected extract; they lose later their species specificity and neutralize the pituitary secretion of the treated animal itself. The changes in the second pair of animals, accordingly, would be due to the injected "antihormones." The result is a state analogous to pituitary insufficiency, with atrophy of the gonads, thyroids and adrenals. The latter leads to cytologic changes in the anterior lobe of the pituitary as described.

I. Davidsohn.

Idiopathic Familial Lipemia. L. E. Holt Jr., F. X. Aylward and H. G. Timbres, Bull. Johns Hopkins Hosp. **64**:279, 1939.

A girl of 11 years was found to have extreme lipemia—due primarily to neutral fat—associated with hepatomegaly, splenomegaly, psoriasis, peculiar ulcerations of the skin and malnutrition. She suffered from periodic acute attacks of abdominal pain, which were accompanied by a sudden reduction in the blood fat, enlargement of the liver and spleen and engorgement of the abdominal veins. There was evidence that the disorder was familial. The condition was not improved by administering lecithin, choline, thyroxin, insulin, "lipocaic" or an

extract of the anterior lobe of the pituitary. It could, however, be in large measure controlled by a low fat diet. Some evidence was obtained of a beneficial effect from blood transfusion. The authors discuss the various types of disturbance which may give rise to lipemia. They suggest that the condition described is due to a defect in the mechanism of the removal of blood fat by the liver—a mechanism in which a humoral factor appears to be concerned.

FROM AUTHORS' SUMMARY.

RADIOACTIVE IRON AND ITS METABOLISM IN ANEMIA. P. F. HAHN, W. F. BALE, E. O. LAWRENCE and G. H. WHIPPLE, J. Exper. Med. **69**:739, 1939.

Artificially produced radioactive iron is an extremely sensitive agent for use in studying the course of iron through its changes in body metabolism, the radioactive iron lending itself to observations of absorption, transport, exchange, mobilization and excretion. The need of the body for iron determines in some manner the absorption of this element. In a normal dog in which there is no need for the element it is absorbed in negligible amounts. In an anemic animal it is promptly assimilated. The plasma is clearly the means of transport of iron from the gastrointestinal tract to the point of mobilization of the element for fabrication into hemoglobin. The speed of absorption and of transfer of iron to the red cell is spectacular. The importance of the liver and of the bone marrow in the metabolism of iron is confirmed.

FROM AUTHORS' SUMMARY.

EXTRAVASCULAR DEVELOPMENT OF THE MONOCYTE OBSERVED IN VIVO. R. H. EBERT and H. W. FLOREY, Brit. J. Exper. Path. **20**:342, 1939.

Ebert and Florey, using a modified Sandison-Clark cell, have watched the extravascular development of individual monocytes into cells indistinguishable from tissue histiocytes. They produce evidence to show that macrophages appearing in organizing tissue do not originate from the histiocytes previously present in surrounding normal tissue.

INTESTINAL REACTIONS TO GASTRIC JUICE. H. W. FLOREY and others, J. Path. & Bact. **49**:105, 1939.

Various technical procedures are described for comparing the resistance of the duodenum to gastric juice with that of the jejunum and ileum, and it is shown that the duodenum has a much greater resistance than other parts of the intestine. These findings are consistent with the view that Brunner's glands are concerned in a mechanism protective against gastric juice. Histologic and endoscopic observations on parts of the intestine exposed to gastric juice are described.

FROM AUTHORS' SUMMARY.

TRANSPLANTATION OF THE TESTICULAR AND THE OVARIAN FATTY BODY. F. X. HAUSBERGER, Virchows Arch. f. path. Anat. **302**:640, 1938.

In the rat at birth there is present beside the head of the epididymis or beside the ovary a small collection of cellular tissue which later becomes the testicular or the ovarian fatty body. At birth it consists of a syncytium of fibrocytes and histiocytes with few blood vessels. On the fifth to sixth day the tissue becomes more highly vascularized, and proliferation leads to the formation of localized cellular areas, in which fat begins to be deposited on the seventh day. The fat is deposited in the form of minute droplets in cells of the histiocyte type, but only in certain of these. In the adult the fatty body is a characteristically lobulated, pedunculated mass of adipose tissue, which is larger in the male than in the female. To study the development of adipose tissue, the fatty bodies of rats 2 to 3 days old were transplanted into the peritoneal cavities of rats weighing 50 to 80 Gm. Only the pedicle of the minute fatty body was sutured to the peritoneum. The transplanted fatty bodies grew and developed exactly as in the normal animals.

Fat was deposited in the cells; the bodies took on the characteristic lobulation and reached a size equal to that of the normal fatty body. The fully developed transplanted male fatty body reached a size larger than that of the female body, no matter whether it was transplanted into a male or a female rat. Transplanted connective tissue of young rats was not transformed into adipose tissue. The author concludes that adipose tissue develops from cells specifically differentiated for that function, probably through the development of specific intracellular enzymes.

O. T. SCHULTZ.

Pathologic Anatomy

THE LYMPHOCYTE IN ACUTE INFLAMMATION IN THE RABBIT. F. KOLOUCH JR., Am. J. Path. **15**:413, 1939.

A transformation of clasmatocytes to histogenous macrophages is the initial response of the rabbit in acute inflammation. The majority of the macrophages in the exudate associated with the acute inflammatory process are of hematogenous origin. The lymphocyte-macrophage transformation occurs early in the course of the inflammation. By the fourteenth hour the lymphocytic origin of many mononuclear cells in an inflamed area is largely obscured. In studies made eighteen hours or later after the onset of an acute inflammation in a tissue, cell lineage cannot be traced. The employment of tissue spreads, dried and stained like blood smears, allows a comparison of the cells in an acutely inflamed tissue with cells of blood smears.

FROM AUTHOR'S CONCLUSIONS.

THE PITUITARY IN MONGOLISM. C. E. BENDA, Arch. Neurol. & Psychiat. **42**:1, 1939.

Benda considers mongolism a pituitary disorder resulting from deficiency of the basophilic and chief cells of the anterior lobe of the hypophysis. The cells are reduced in number, with the result that there is an apparent increase of the eosinophilic cells. Benda arrived at this conclusion from a study of 14 patients, in 13 of whom mongolism was unquestionably present. The histologic changes were identical regardless of the age (from a few days to 30 years) and were the reverse of what is seen in Cushing's disease, in which the number of the basophilic cells is increased. The condition of the thyroid has no bearing on the histologic changes in mongolism.

GEORGE B. HASSIN.

FATAL HYPOGLYCEMIA. A. L. SAHS and L. ALEXANDER, Arch. Neurol. & Psychiat. **42**:285, 1939.

In a fatal case of hypoglycemic shock of twenty-eight hours' duration, the benzidine staining method revealed a number of anomalies in the blood vessels and capillaries—hyperemia, irregular dilatation, perivascular hemorrhages, variations in the appearance of the capillaries and blood vessels, thrombi and so-called "white stasis," i. e., early thrombosis in which white blood cells are enmeshed in scanty threads of fibrin. Ganglion cells showed ischemic degeneration, and in the cortex of the central and upper parietal regions there were foci of blanching. The nerve fibers were well preserved, but the axons were "pushed apart as though by interstitial edema." The authors consider the blanching, the edema and the changes in the ganglion cells secondary to the vascular changes and due to local and general anoxia.

GEORGE B. HASSIN.

TEETH IN OSTEOGENESIS IMPERFECTA. M. A. RUSHTON, J. Path. & Bact. **48**:591, 1939.

Teeth from a woman who had suffered from late osteogenesis imperfecta are described. They were small, with pinkish crowns and short translucent roots. The odontoblasts had differentiated normally, though the growth of the whole dentinal

papilla was reduced. Normal matrix and Tomes' fibrils were produced at the periphery of the teeth, but soon the odontoblasts and other cells concerned ceased to form Tomes' fibrils and normal matrix. This change occurred first where the odontoblasts were oldest, but in a piecemeal manner. Focal defects in the rate of formation of the matrix led to the inclusion therein of blood vessels. The peripheral pulp cells produced precollagenous argyrophil fibers, but these were not converted into collagen except in the immediate proximity of blood vessels. Pulp stones of good collagen content occurred in the middle of the dentinal papilla. The matrix was inadequately calcified and probably lacking in cementing substances. At a later stage, when no normal Tomes' fibrils were being formed, a tissue resembling primitive fiber bone replaced the former pulp cavity. Most of the dentinal tubules which had been formed became occluded. FROM AUTHOR'S SUMMARY.

EFFECT OF ACUTE AND CHRONIC URINARY RETENTION ON THE KIDNEY. K. HELMKE, Virchows Arch. f. path. Anat. **302**:323, 1938.

As the result of a histologic study, Helmke reaches the conclusion that chronic stasis of urine has an effect on the kidney quite different from that of acute retention. The latter causes single or multiple ruptures of the calices in the region of the fornix and leads to a reflux of urine directly into the veins of the pelvis. The escape of urine into the blood stream may lead to acute collapse. The path of escape may be detected roentgenologically. If infection does not occur, the process heals when the cause of the acute retention is relieved. In urinary retention of more gradual onset and longer duration the retained urine distends the glomerular capsules and escapes into the interstitial tissue of the kidney. From here it makes its way into the lymphatics and finally into the veins. In the veins and lymphatics the material has the same optical and staining properties as in the tubules and glomerular capsules; Helmke terms these masses venous and lymphatic cylinders or casts. The constituent common to tubular casts and venous cylinders is urinary mucoid. The veins and lymphatics of the interstitial tissue play an important part in the spread of ascending infections of the kidney.

O. T. SCHULTZ.

HISTOLOGY OF CHRONIC PEPTIC GASTRIC AND DUODENAL ULCER. A. THELEN, Virchows Arch. f. path. Anat. **302**:515, 1938.

A histologic study of gastric and duodenal ulcer was made in 32 resection specimens by a variety of staining methods. Especial attention was paid to the formation and composition of the characteristic zone in the floor of the ulcer to which Askanazy had given the name "layer of fibrinoid necrosis." In recent years Klinge and many others have applied the term "fibrinoid" to a change in connective tissue, occurring usually in hyperergic inflammation, characterized by transformation of connective tissue into fibrillated argentophil material. In this sense Askanazy's layer of fibrinoid necrosis is not the result of fibrinoid degeneration. The layer is formed by swelling of the connective tissue without the formation of fibrinoid, the swollen fibers undergoing necrobiosis. The author terms this process *Quellungsnekrose* (swelling necrosis). On the surface of the layer of necrosis there may be deposited a layer of exudate composed of true fibrin, leukocytes and erythrocytes; beneath the zone of necrosis is the zone of proliferative inflammatory reaction. The layer of necrosis may be demarcated and sequestrated, a new necrotic layer being formed. The acid gastric secretion is a factor in the formation of the necrotic layer, but apparently not necessarily directly, since an ulcer may reveal necrosis in one portion and healing in another. Thelen postulates a disturbance of the correlation gastric juice: gastric wall.

O. T. SCHULTZ.

Microbiology and Parasitology

TUBERCLE BACILLI IN NON-TUBERCULOUS LUNG TISSUE. W. H. FELDMAN and A. H. BAGGENSTOSS, Am. J. Path. **15**:501, 1939.

The results of this study, which was made of material from an area where the morbidity from tuberculosis is not high, indicate that virulent tubercle bacilli are infrequently present in the presumably nontuberculous tissue of the lungs of persons dying of causes other than tuberculosis. FROM AUTHORS' SUMMARY.

INTRACELLULAR BACILLI IN TYPHOID FEVER. J. W. ADAMS JR., Am. J. Path. **15**: 561, 1939.

Gram-negative bacillary forms, judged to be Eberthella typhi, have been found in the cytoplasm of young plasma cells located in the lymphoid follicles of the ileum, colon and mesenteric lymph nodes in 5 cases of early typhoid fever. It is concluded that the presence of these bacillary forms within the plasma cell is an essential part of the early classic intestinal and mesenteric lesions of typhoid fever.

FROM AUTHOR'S SUMMARY.

EFFECT OF ULTRAVIOLET RADIATION ON TUBERCLE BACILLI. K. C. SMITHBURN and G. I. LAVIN, Am. Rev. Tuberc. **39**:782, 1939.

The effect of approximately monochromatic ultraviolet radiation (2,537 angstroms) on human tubercle bacilli in saline suspension was studied. Heavy suspensions of tubercle bacilli (1 mg. per cubic centimeter) required relatively long periods of irradiation (ten minutes or more) before the organisms were rendered nonviable; weaker suspensions, a shorter time. Organisms killed by ultraviolet radiation retained the property of acid-fastness. Bacilli in heavy suspensions were rendered avirulent only after relatively long exposure to ultraviolet radiation, but those in weak suspensions were quickly reduced in virulence. Reduction in virulence could be demonstrated after less irradiation than is required to kill the organisms, and the organisms could be avirulent without being killed. Irradiated viable organisms possessed the capacity of inducing demonstrable immunity. Organisms killed by the radiation did not induce measurable immunity.

H. J. CORPER.

VITAMIN C AND IMMUNITY IN TUBERCULOSIS OF GUINEA PIGS. F. H. HEISE and W. STEENKEN JR., Am. Rev. Tuberc. **39**:794, 1939.

Vitamin C given subcutaneously and in abundance does not influence the course of tuberculosis in guinea pigs infected with 10,000 H 37 *Rv* bacilli, does not influence the content of vitamin C in the blood serum and does not influence the sensitivity to tuberculin. Rotter's test proved of no value in differentiating supervitaminosis C.

H. J. CORPER.

PRESERVATION OF TUBERCLE BACILLI. M. L. COHN, Am. Rev. Tuberc. **40**:99, 1939.

Desiccated human (virulent and avirulent), bovine (virulent) and avian (virulent) tubercle bacilli at refrigerator temperature retain their viability almost completely for three years, while natural cultures at the same temperature survive only about six months to one year (occasionally two years). At incubator temperature the loss of viability of the desiccated cultures of these same strains of tubercle bacilli is much more rapid, being almost complete at six months to one year, while at room temperature such cultures survive a little longer than at incubator temperature. The loss of viability of tubercle bacilli is primarily a function of the temperature at which they are stored regardless of whether they are desiccated or natural. The rate of the loss of viability of cultures is greater in the presence of oxygen, less in the presence of air and least in the presence of nitrogen. Desiccation, although a minor factor, aids in maintaining the viability. Desiccation can

remove as much as 70 to 75 per cent of the water (and volatile materials) from the cultures without appreciable detrimental effect on the viability. Aside from mammalian bacilli, cultures of avian tubercle bacilli can be preserved in viable form for over three years when desiccated and sealed in air or nitrogen and maintained at refrigerator temperature. The method possesses the advantage that the strains occupy little space and are readily transportable. H. J. Corper.

The Spread of Tubercle Bacilli in Sensitized and Immunized Animals. J. Freund and D. M. Angevine, J. Immunol. **35**:271, 1938.

Rabbits were given several intracutaneous injections of heat-killed tubercle bacilli and were tested for hypersensitiveness one week after the last injection of old tuberculin. They were then inoculated intradermally with living tubercle bacilli, bovine or human, of varying degrees of virulence. Normal rabbits were similarly inoculated with living tubercle bacilli at selected intervals. Immunized and control rabbits were killed, and the skin at the site of injection and the inguinal nodes were removed and examined for tubercle bacilli culturally and histologically. In the previously immunized animals the tubercle bacilli multiplied and were more abundant in the skin after the same interval than in the infected animals that were previously normal. In the immunized animals the bacilli reached the regional lymph nodes from two days to two weeks later than in the normal controls. It appears that this retardation was caused by local fixation of the bacilli and not by destruction. I. Davidsohn.

Purification of Insect-Transmitted Plant Viruses. F. C. Bawden and N. W. Pirie, Brit. J. Exper. Path. **20**:322, 1939.

From plants infected with potato virus Y, which is an insect-transmitted virus, Bawden and Pirie have isolated a liquid crystalline protein with many properties similar to those of potato virus X, although it is much less stable. The isolation is complicated by the small amount of virus in infective sap and by the presence of other constituents having properties in common with the virus.

Action of Bile Salts on Viruses. W. Smith, J. Path. & Bact. **48**:557, 1939.

Certain bile salts are able to inactivate some viruses but have no apparent effect on others. The inactivation is almost instantaneous and is thought to depend on lysis of the virus elements. Sodium deoxycholate and sodium apocholate are the most active of the bile salts investigated, the former being about four times as active as the latter. Sodium cholate possesses only slight activity. The susceptibility of a virus to bile salts is not related to its size. The process of lysis by a bile salt can be observed both macroscopically and microscopically with the cultivable virus-like organism of pleuropneumonia and the sewage organisms of Laidlaw and Elford. Attempts to use preparations of lysed virus for prophylactic immunization have so far not been encouraging. From Author's Summary.

Tuberculous Superinfections. V. Reynes, Ann. Inst. Pasteur **62**:177, 1939.

Reynes studied the reactions which appear in superinfected guinea pigs from the eleventh to the eighty-sixth day after the first inoculation with fragments of autogenous bacillary lesions (autosuperinfections) or fragments of tuberculous lesions developing on other animals (heterosuperinfections). Contrary to descriptions by other authors, he shows that the lesions and reactions were the same in these guinea pigs whether the animals were tested with their own organisms or with those of other animals. Superinfections occurring in the three weeks following the primary inoculation develop like primary infections and may lead to the production of a local lesion of superinfection if the quantity of bacilli is sufficient. Superinfections occurring a month after primary inoculation are usually without effect. Test organisms are then either destroyed or blocked in the tissues in which they are deposited or in which the culture may be found after a longer

or shorter period. Sometimes, also, the immunity created by a primary infection is indicated only by an obstacle to the dissemination of the inoculated organisms; there is then a longer lapse of time before the ganglions in the superinfected animal are attacked than in the control animal. Finally, this immunity may be forced when the material inoculated contains a large number of bacilli; i. e., the organisms not only multiply where inoculated but reach the neighboring lymph nodes and produce lesions.

FROM AUTHOR'S SUMMARY.

Immunology

PRODUCTION OF KIDNEY ANTIBODIES. F. F. SCHWENTKER and F. C. COMPLOIER, J. Exper. Med. **70**:223, 1939.

Rabbits given injections of emulsions of homologous kidney to which staphylococcus or streptococcus toxins had been added produced complement-fixing antibodies which reacted with both rabbit kidney and brain. By absorption tests it was demonstrated that the serum contained at least two antibodies, one specific for kidney and the other nonspecific. Similar antibodies for kidney were found in the blood of a majority of patients with scarlet fever but in the blood of only a few normal persons. The possibility that a similar or related antibody may be etiologically concerned in scarlatinal nephritis is discussed.

FROM AUTHORS' SUMMARY.

SPECIFIC ANTISERUM FOR BLOOD-GROUP FACTORS A, B, M AND N. W. C. BOYD, J. Immunol. **37**:65, 1939.

Satisfactory anti-A agglutinating serum was produced relatively easily in rabbits, i. e., in somewhat less than 50 per cent of the animals, by inoculating them with human A red cells. The serum was absorbed to remove the anti-B, anti-M and anti-N agglutinins. Anti-B serum was also produced but not quite as easily. In an attempt to produce anti-A and anti-B heteroagglutinins by injecting other antigens than human red cells into animals, rabbits and roosters were inoculated with human or horse saliva containing blood group factors A or B, with peptone (Witte) containing factor A and with rabbit red cells known to contain a fraction of the human factor B. In response to human saliva A and to peptone, highly group-specific agglutinins were produced. The anti-B serum was less satisfactory. Methods of preservation of serum, including the anti-M and anti-N testing fluids, are offered which made it possible to keep them in usable strength for as long as five and one-half years.

I. DAVIDSOHN.

IMMUNOLOGICAL RELATIONSHIP OF THE VIRUS OF SPONTANEOUS COWPOX TO VACCINIA VIRUS. A. W. DOWNIE, Brit. J. Exper. Path. **20**:158, 1939.

Downie's finding that histologic differences occurred in the lesions produced by the viruses of cowpox and vaccinia led him to investigate these two viruses by serologic methods. He now shows that rabbits immunized with either virus are immune to both and that immune serum prepared with either virus neutralizes both. However, cross absorption experiments with hyperimmune serum and suspensions of elementary bodies show that while the homologous virus removes the antibodies for both viruses, the heterologous virus absorbs chiefly its own antibodies. These results indicate that the viruses of cowpox and vaccinia are closely related but not identical as previously assumed by many workers.

ACTION OF PROTEOLYTIC ENZYMES ON THE ANTITOXINS AND PROTEINS IN IMMUNE SERUM. C. G. POPE, Brit. J. Exper. Path. **20**:201, 1939.

In the second paper dealing with the action of proteolytic enzymes on antitoxic serum Pope describes the results obtained after a limited amount of enzyme action on antitoxins followed by critical heat denaturation of nonantitoxic protein.

Several enzymes possess the property of altering the antitoxic pseudoglobulin and converting it into two protein fractions, one of which is nonantitoxic and easily denatured in acid solutions at high temperatures. The antitoxin is not destroyed by this treatment.

COMPLEMENT IN NEPHRITIS. C. E. KELLETT and J. G. THOMSON, J. Path. & Bact. **48**:519, 1939.

In 38 cases of nephritis the complementary activity of the serum for sensitized sheep cells was estimated. The cases were subdivided into groups on clinical grounds and the classification confirmed when possible by autopsy. In every case of acute glomerulonephritis examined within four weeks after the onset complementary activity was found to be much lower than normal.

FROM AUTHORS' SUMMARY.

Tumors

INFLUENCE OF DINITROKRESOL ON THE DEVELOPMENT OF TAR TUMORS IN MICE. L. KREYBERG, Am. J. Cancer **36**:51, 1939.

Sixty mice of a genetically known strain have been painted with tar and treated with large doses of dinitrocresol (from 0.1 to 2 mg. per day). The tumor response corresponds closely to that of similar animals treated with dried thyroid gland, namely, a general tendency to earlier formation of tar tumors.

FROM AUTHOR'S SUMMARY.

EFFECT OF HYPOPHYSEAL TRANSPLANTS IN VARIOUS STRAINS OF MICE. L. LOEB and M. M. KIRTZ, Am. J. Cancer **36**:56, 1939.

The processes of normal and cancerous growth which take place in bearers of anterior hypophysial transplants are in certain respects more similar to those taking place spontaneously in mice than to those induced by injections of estrogen. In both the so-called spontaneous tumors and those induced by transplanting anterior lobe of the hypophysis the hormones which represent the effective stimuli originate in the animals themselves, whereas in tumors induced by injecting estrogen the source of effective stimulation is extrinsic.

FROM AUTHORS' SUMMARY.

MORTALITY FROM CANCER OF THE SKIN. K. K. CONRAD and A. B. HILL, Am. J. Cancer **36**:83, 1939.

A comparison is made between the mortality from cancer of the skin and lip and that from cancer of other sites in different occupational groups. The reported inverse association between these forms of cancer is not confirmed. On the contrary, although numerous exceptions occur, there is on the average a slight direct association, occupations in which there is a relatively high incidence of cancer of the skin and lip tending to show also an excess of cancer of other sites.

FROM AUTHORS' SUMMARY.

ASPIRATION BIOPSY OF TUMOR OF THE LIVER. J. S. BINKLEY, Am. J. Cancer **36**:193, 1939.

Aspiration biopsy for tumor of the liver has been done in 19 cases. Sufficient material was obtained by aspiration to establish a diagnosis in 73.6 per cent of the cases. Follow-up records of the clinical course in each case support the histologic diagnosis. Aspiration biopsy of hepatic tumors has not been associated with significant complications in the hands of 10 different clinicians who have used the method on one or more occasions.

FROM AUTHOR'S SUMMARY.

STUDIES IN CARCINOGENESIS: HYDROCARBON-CHOLESTEROL PELLETS IN ALBINO MICE. M. J. SHEAR and EGON LORENZ, Am. J. Cancer **36**:201, 1939.

Cholesterol pellets containing 5 per cent dibenzanthracene produced tumors in the course of a year in a considerable proportion of the mice in which they were implanted. Similar pellets containing 1 per cent dibenzanthracene produced tumors in only a small percentage of mice. The two 1 per cent pellets which produced tumors contained 0.12 mg. and 0.48 mg. of dibenzanthracene, respectively. Spectrographic analysis of these pellets, recovered after the induction of tumors, showed that there was still some dibenzanthracene present. No tumors were obtained with cholesterol pellets containing 0.1 and 0.01 per cent, respectively, of dibenzanthracene. The significance of the one sarcoma obtained with a 0.001 per cent dibenzanthracene–cholesterol pellet is therefore obscure.

FROM AUTHORS' SUMMARY.

STUDIES IN CARCINOGENESIS: COMPOUNDS RELATED TO 3,4-BENZPYRENE. M. J. SHEAR, Am. J. Cancer **36**:211, 1939.

Twelve derivatives of 3,4-benzpyrene were tested for carcinogenic potency by injecting them subcutaneously into pure strain mice. Of these derivatives, the 4′-methyl and the 1′,2′-dihydro-4′-methyl derivatives were found to be carcinogenic. The results obtained with 80 other polycyclic compounds are briefly recorded. The findings are discussed from the point of view of the relationship of these synthetic compounds to substances of biologic origin and from that of the implications as regards the mechanism of tumor genesis.

FROM AUTHOR'S SUMMARY.

EXPERIMENTAL ZINC TERATOMA OF THE TESTIS. L. I. FALIN and K. E. GROMZEWA, Am. J. Cancer **36**:233, 1939.

The injection of small amounts of 10 per cent zinc sulfate solution (0.15 to 0.2 cc.) into the genital glands of fowl produces in a considerable percentage (15 per cent of all birds treated in March) rapidly growing tumors of these glands. Being composed of a great variety of tissues—epithelial and glandular elements, cartilage, developing bones, smooth muscle tissue, pigment cells, embryonic connective tissue, anlagen of nerve elements—these tumors must be classified as teratoid neoplasms and in this respect are quite similar to the teratoma produced by Michalowsky and others with zinc chloride. The production of teratoid tumors of the testes by injections of either zinc chloride or zinc sulfate solution shows that the ions Cl_2 and SO_4 do not play any particular role in the production of tumors by zinc salts in the genital glands of fowl. Further experiments must show whether other substances having a similar influence on the tissues but having nothing in common with the zinc salts in their chemical structure may produce a similar effect. (See also the article on experimental zinc teratoma of the testis by V. Anissimova [*Am. J. Cancer* **36**:229, 1939].)

FROM AUTHORS' SUMMARY.

ANGIORETICULOENDOTHELIOMA (KAPOSI'S DISEASE) OF THE HEART. R. M. CHOISSER and E. M. RAMSEY, Am. J. Path. **15**:155, 1939.

The problem of the origin and of the nature of the lesions of Kaposi's disease has been reanalyzed on the basis of a review of the literature on the subject and in the light of a thorough study of 2 recent cases, in both of which the disease was primary in the right auricle of the heart and in which lesions of the skin were lacking. It is concluded that the condition is a true neoplasm, derived from the reticuloendothelial system, with neoformation of blood vessels a prominent distinguishing characteristic. Since tumors are properly named with respect to their tissues of origin, it is proposed that in the future the scientific term "angioreticuloendothelioma" be used in preference to the term "Kaposi's disease."

FROM AUTHORS' SUMMARY.

CARCINOMA OF THE PANCREAS. R. D'AUNOY, M. A. OGDEN and B. HALPERT, Am. J. Path. **15**:217, 1939.

In 6,050 autopsies on persons over 1 year of age 40 cases of primary carcinoma of the pancreas were encountered. Males and females were represented in the series in the proportion of 7:1. Twenty-three of the patients were over 60 years of age. The average duration of illness was four and a half months. Thirty-one neoplasms were situated in the head and 9 in the tail. All were columnar cell carcinoma. Carcinoma primary in the head of the pancreas readily invaded the duodenum, and that primary in the tail spread over the peritoneum. Metastases were observed in the liver in 25 instances.

FROM AUTHORS' SUMMARY.

FOLLICULAR LYMPHOBLASTOMA AND A RELATED FORM OF LYMPHOSARCOMA. S. MAYER JR. and H. M. THOMAS JR., Bull. Johns Hopkins Hosp. **64**:315, 1939.

Five cases of follicular lymphoblastoma are reported. In this series follicular lymphoblastoma has not proved to be entirely benign, despite the usual dramatic early response to radiotherapy. The length of response to irradiation cannot be prophesied from the presence or absence of microscopic evidence of invasiveness. The possibility of clinical recognition of follicular lymphoblastoma is emphasized. The authors describe 4 cases of malignant lymphoblastoma of an unusual but characteristic type. The patients presented the following features: general or local lymphadenomegaly; splenomegaly due to the presence of tumor cells, which were localized especially in the malpighian bodies; lymph nodes showing diffuse infiltration with lymphoblastic cells; a normal blood picture except for moderate anemia; a rapidly fatal course.

FROM AUTHORS' SUMMARY.

INTESTINAL POLYPS: GENESIS AND RELATION TO MALIGNANT GROWTHS. R. J. COFFEY and J. A. BARGEN, Surg., Gynec. & Obst. **69**:136, 1939.

A study of a group of cases of multiple adenomatosis and of polyposis associated with chronic ulcerative colitis revealed a striking dissimilarity both clinically and pathologically. Of the cases of multiple adenomatosis, a heredofamilial disposition was present in 34.5 per cent. It is essentially a disease of youth, approximately two thirds of the patients being in the first three decades of life. Instances of the condition developing in later life were not uncommon, and the growths were indistinguishable from those in adolescents. The individual lesion consisted of a primary epithelial change with minimal evidence of inflammation. Hypertrophic lymph follicles seemed to have a part in the genesis of the polyps. Characteristic of the condition are myriads of true adenomatous polyps. Carcinomatous transformation was demonstrable in 62.5 per cent; in 25 per cent multiple carcinomas occurred. Histologically it appeared that multiple small foci of adenomatous proliferation develop in a usually hyperplastic mucosa. For this reason the condition is a disease of the entire mucosa, and eradication of only the existent polyps fails to cure the process.

Polyposis complicating chronic ulcerative colitis is characterized by widespread inflammation and destruction of the mucosa with inflammation of the whole wall. The polyps consist of tufts of granulation tissue and of surviving remnants of mucosa, commonly showing benign regenerative hyperplasia, often with adenomatous or even carcinomatous transformation. In this group 56.2 per cent were classed as pseudoadenomatous, 21.9 as true adenoma and 21.9 per cent as carcinomatous. Polyps associated with chronic ulcerative colitis seem to be the result of widespread ulceration and destruction of the mucosa, associated with remaining islets of inflammatory mucous membrane, followed by cicatricial distortion of the lining. It seems that multiple adenomatosis is potentially a much more dangerous condition with respect to carcinoma than polyposis associated with chronic ulcerative colitis.

FROM AUTHORS' SUMMARY (WARREN C. HUNTER).

TWO RARE TUMORS OF THE THYROID. UMEDA, Virchows Arch. f. path. Anat. **302**:458, 1938.

The tumors described are a papillary carcinoma, which occurred in the thyroid of a 7 year old boy, and a fibro-osteochondrosarcoma, which occurred in a woman aged 68. Skeletal metastasis did not occur in either case, and evidences of thyroid deficiency were not observed.

O. T. SCHULTZ.

DAMAGE OF LUNGS FOLLOWING ROENTGEN IRRADIATION. H. VOEGT, Virchows Arch. f. path. Anat. **302**:468, 1938.

In a patient with mammary carcinoma and another with Hodgkin's disease, both of whom had been subjected to therapeutic roentgen irradiation of the chest, marked fibrosis of the lungs in the areas previously irradiated was found at autopsy. This is considered the end stage of damage done to the lungs at the time of irradiation.

O. T. SCHULTZ.

EXTRAMEDULLARY PLASMOCYTOMA. H. VOEGT, Virchows Arch. f. path. Anat. **302**:497, 1938.

Five examples of extramedullary plasmocytoma and 1 example of plasmocytic granuloma are described. Of the 5 neoplasms, 3 arose in the upper air passages, 1 in the maxillary antrum and 1 in the thyroid (the first recorded instance of such a tumor in this organ). The true tumors are composed of cells of a single type, the plasma cell, which may reveal considerable pleomorphism.

O. T. SCHULTZ.

Medicolegal Pathology

THE DURET-BERNER HEMORRHAGES. B. DAHL, Deutsche Ztschr. f. d. ges. gerichtl. Med. **29**:366, 1938.

Berner, in his early observations on hemorrhages occurring in the floor of the fourth ventricle after injuries to the head, believed these hemorrhages impaired the function of the vital medullary centers and directly caused death. In 1936 he questioned the importance of such hemorrhages as lethal agents and suggested that they might be meaningless agonal collections of blood resulting from diapedesis. Duret produced hemorrhages in the floor of the fourth ventricle and in other parts of the brain by injecting quickly into the subdural space a large volume of liquid. The similarity of the hemorrhages observed by Duret and Berner under widely different circumstances led to the employment of the hyphenated name "Duret-Berner" to designate a special type of extravasation. In investigating hemorrhages of this nature, Dahl chose 50 corpses in widely divergent groups as concerned terminal illness and mode of death.

The subjects in the first group died suddenly from coronary sclerosis, pulmonary emboli, convulsions with epilepsy, gunshot wounds, hanging, acute alcoholism and strychnine poisoning. The second group was afflicted with acute illnesses, such as septicemia, otitis media, hemorrhagic diathesis and acute infections and intoxication. The third group died of chronic illnesses, such as carcinoma, sarcoma, tuberculosis, pernicious anemia and chronic cardiac and renal ailments. The results of the studies of the brains in the three groups were strikingly similar. Hemorrhages occurred regularly in the pia, especially in the sulci, where small collections of blood apparently were due to diapedesis and larger collections came from actual tears in the venules, which could be demonstrated in serial sections.

Rows of veins were grossly visible in the floor of the fourth ventricle in front of the stria acoustica. These veins had an extra-wide perivascular space which had to be traversed by tributary venules. In the vicinity of these veins ring

hemorrhages were most common. They drained to a focal point in the pons, and the resulting cluster did not allow all the veins to empty at once. As a result, some veins became overdistended and ruptured. Similar hemorrhages were found in the lung, mesentery and other parts of the body. As a rule, quick death is associated with numerous hemorrhages in the brain, as are ailments which lower the resistance of the veins—e. g., leukemia, hemorrhagic diathesis and anaphylactic shock. Still other conditions cause hemorrhages by an increase in venous pressure as encountered in strangulation, forcible respiration and compression of the abdomen.

Experiments on guinea pigs and rabbits failed to produce hemorrhages in the brain similar to those seen in human beings. An explanation for this is found in the fact that in these animals the veins in the vicinity of the fourth ventricle are distributed evenly and do not cluster in a pontile knot as do the veins in man. Dahl believes that the hemorrhages observed by Berner and Duret are not the same. He contends that the Berner hemorrhages are not the cause of death but are the result of the true cause of death, as are agonal hemorrhages elsewhere.

GEORGE J. RUKSTINAT.

CEREBRAL CHANGES IN CASES OF SUDDEN DEATH. A. WELZ, Virchows Arch. f. path. Anat. **302**:657, 1938.

In 4 cases of sudden death no adequate gross cause of death could be discovered at necropsy. The persons had previously been apparently healthy. Microscopic examination revealed a form of encephalitis characterzed by perivascular lymphocytic infiltration of the brain and meninges. The condition is not believed to be a specific form of encephalitis. The author's concept is that some previous infection had altered the reactivity of the vessels, causing them to react by a cellular exudative process to some factor, such as trauma, anesthesia, intoxication or infection, insufficient in itself to cause death. The end effect is looked on as a cerebral circulatory death.

O. T. SCHULTZ.

Society Transactions

NEW YORK PATHOLOGICAL SOCIETY

ALFRED PLAUT, *President*

CHARLES T. OLCOTT, *Secretary*

Regular Meeting, Nov. 30, 1939

Subacute Endocarditis with Systemic Moniliosis. S. H. POLAYES.

A white man 48 years of age, a drug addict for twenty years and, in the parlance of drug addicts, a "main line shooter" (inoculating himself intravenously) was brought into the Cumberland Hospital, of Brooklyn, April 24, 1939, complaining of epigastric pain, vomiting and fever of two days' duration. Since January 1939 he had been suffering from pains in joints and muscles and was gradually becoming weaker. He had had gonorrhea twenty-nine years before, diphtheria twelve, a cardiac murmur four and pneumonia two years before. His father died of tuberculosis.

This man presented evidence of chronic illness, a "spiking" temperature, a pulse rate of 114 and 28 respirations per minute. The blood pressure was 90 systolic (there is a question as to the diastolic pressure). There were blue spots in the skin along the veins of the arm. A rough aortic systolic murmur was heard. A roentgenogram of the heart showed a mitral configuration. There was abdominal tenderness to deep palpation. The skin showed crops of petechiae. There were transitory cutaneous nodules in the palms and soles. There were splenic enlargement, sternal tenderness, pains in joints and chills. Death followed seven weeks after admission.

During the course of this man's illness each of thirteen blood cultures showed pure colonies of monilia. The rest of the laboratory data are not pertinent. A biopsy of the skin through the blue specks revealed a foreign body granulomatous reaction caused by iron-free particles of debris (probably introduced with the needle).

On postmortem examination the heart showed the most interesting of all the changes. The right posterior cusp of the aortic valve was almost completely replaced by a cauliflower-like mass of vegetations composed of monilia, leukocytes and fibrin. The mass projected 4 cm. upward into the lumen of the aorta; at the base it was continuous with a fluctuating mass of necrotic structure and colonies of monilia, which produced a bulging of the right atrial wall, perforating at a point just above the medial cusp of the tricuspid valve.

The complete list of anatomic diagnoses follows: monilial endocarditis; fibrosis and calcification of the aortic valve; acute interstitial myocarditis and pericarditis; myofibrosis cordis; infarcts of the spleen and kidneys; hemorrhagic pneumonitis; acute hepatitis; meningoencephalitis and hemorrhages of the spinal cord; aneurysm of the superior mesenteric artery; subcutaneous foreign body granulomas of the upper extremities.

DISCUSSION

AMOUR F. LIBER: Was any attempt at therapy made?

S. H. POLAYES: We used sulfanilamide, if that is what you mean.

AMOUR F. LIBER: No, there are several other treatments for mycoses.

Hyperparathyroidism in Infancy and Childhood. DOROTHY H. ANDERSEN (by invitation).

Within recent years patients with hyperparathyroidism have been found to be divisible into two groups: (1) those whose condition is due to a primary adenoma of the parathyroid, and (2) those whose condition is due to hyperplasia of the

parathyroid, secondary to prolonged severe renal insufficiency. In the first group the symptoms may be relieved by parathyroidectomy, but in the second group such relief is only temporary. In both groups there are bone changes of a similar nature, a disturbance of the calcium and phosphorus metabolism and sometimes metastatic calcification. The concept that prolonged renal insufficiency produces hyperplasia and hyperactivity of the parathyroids is on a firm basis. Within recent years it has been recognized that renal rickets is a form of renal hyperparathyroidism occurring in late childhood and bears the same relationship to adult renal hyperparathyroidism that gigantism does to acromegaly.

A case is reported of renal hyperparathyroidism in an infant dying at the age of 6 months of calcification of the arteries, including the coronary arteries. This case presents the essential characteristics of the disease: (1) a severe renal insufficiency, due in this instance to congenital hydronephrosis and cystic kidneys; (2) hyperplasia of the parathyroids; (3) high serum phosphorus and nonprotein nitrogen, low serum calcium and low carbon dioxide-combining power; (4) the bone changes of mild hyperparathyroidism, and (5) metastatic calcification of the middle-sized arteries. Death was apparently due to a coronary infarct. The chief points of difference from the adult disease were the lesser degrees of parathyroid hyperplasia and of bone changes.

Architecture of the Amyloid Kidney. JEAN R. OLIVER.

This presentation consisted of a demonstration of drawings and photographs of microdissections of amyloid kidneys.

The places of deposit of the amyloid in the early and late stages of the disease were shown, and it was emphasized that the deposition of the amyloid cannot be an infiltration which proceeds from certain points in the vascular tree but is a scattering of minute deposits which ultimately fuse. The new formation of vascular branches from the arteries direct to the tubular circulation was also demonstrated.

The distortion of nephrons is similar in character to that seen in other forms of chronic Bright's disease. The processes of hypertrophy and hyperplasia are, as in these other forms, limited to the proximal convolution. Aglomerular tubules are also present. These parenchymal distortions seem to be chiefly the effect of a reactive inflammatory process occurring in the interstitial substance of the organ.

An examination of the topography of the amyloid kidney shows that the same sorts of metallaxis are found in it as in other forms of chronic Bright's disease.

DISCUSSION

PAUL KLEMPERER: I should like to ask whether the preparations came from kidneys which were small. Or were they still large? I ask the question because patients with so-called amyloid contracted kidney rarely have a history of amyloid nephrosis. I wonder whether there are differences in the development of the contraction of the kidney in amyloidosis; therefore I should like to know whether the kidneys were small.

SILIK H. POLAYES: I should like to ask whether it is not possible that some of the infiltrate of the so-called interstitial nephritis which was observed might represent a coincidental infiltration which preceded the deposition of amyloid. I ask that because one finds it so often in the kidney, not related to the pathologic alterations produced in the amyloid kidney.

ALFRED PLAUT: I do not know if Dr. Olver will like my question, but I cannot help it. He has stated that there is no real relation between the morphologic changes in the glomeruli and those of the tubules. I should like to know what Dr. Oliver's opinion is of the contradiction in this demonstrated fact with the widely accepted opinion that the blood supply of the tubules to a large extent has to go through the glomerular loops. Often in cases of glomerulonephritis one sees most of the glomerular loops almost empty, without any blood, and, nevertheless, sees little change in the tubules. I have never been able to reconcile this with the absence of a nonglomerular blood supply to the tubules as stressed by most pathologists and anatomists.

Dr. Oliver has asked a question concerning the word "metallaxis." I think this word should be pronounced as the more familiar term "parallaxis" is pronounced. Both words are derived from the Greek *allatto* (ἀλλαττω), which means to change. It is a good term and might be used not only for the kidney and its vessels. Certain structural changes that take place in the chronically congested liver, for instance, could be termed metallaxis. No other term in English nomenclature is available for this change; the German authors call it *Umbau*.

JEAN R. OLIVER: In answer to Dr. Klemperer's question, I might say that my co-workers and I did not dissect any very small amyloid kidneys. We never had any kidney to which we really wanted to give the name "contracted kidney"; by that I mean a kidney 3 or 4 cm. in longest dimension. All of these specimens were taken from kidneys of about normal size. Most were somewhat under normal, but they were not severely contracted. They were all kidneys, for example, which one could easily determine grossly to be amyloid kidneys. My answer to his question cannot be very direct.

As to whether the inflammation which is found in these sections might be preexistent inflammation, I do not know any way to deny it. As Dr. Polayes points out, one finds such inflammation very commonly in all sorts of kidneys, but I don't think that means it has nothing to do with the parenchymal deformity that one finds in the same kidneys, because that also is very common. I do not think there is anything specific or peculiar or primary or unique in the inflammatory reaction one finds in the interstitial tissue of the amyloid kidney; nevertheless that reaction is there, and I cannot see how it can help but produce changes in the parenchymal structures.

The matter of the blood flowing through the glomeruli before reaching the tubules, which Dr. Plaut brought up, is one of those considerations that have been disturbing, as he says, to pathologists. They look at the glomeruli and cannot see any patent blood vessels in them, and yet they have been taught that this is the only way that blood can get to the tubules. I have no doubt that in abnormal kidneys there are many ways that blood can get to the tubules other than through the glomeruli. Dr. Plaut mentions the Ludwig vessels. There are also direct branches from the interlobular artery, and there are arteriovenous connections or anastomoses that allow the blood to flow from the artery into the vein, then backward out around the tubules. It never seemed to me that this last way was a very efficient way of irrigating the kidney, but it might help. When one looks at a section and sees occlusion of glomerular capillaries, one should remember that one is looking at only a few and that the condition of the capillaries varies in normal kidneys, some being patent and some shut. It is therefore difficult to estimate the state of the circulation of the kidney by looking at a few sections of a few glomeruli.

I am thankful to Dr. Plaut for the philologic discussion; it never occurred to me that the word "metallaxis" was similar to "parallaxis"; of course, it is.

CHICAGO PATHOLOGICAL SOCIETY

S. A. LEVINSON, *President*

EDWIN F. HIRSCH, *Secretary*

Regular Monthly Meeting, Dec. 11, 1939

Experimental Retinoblastoma. ARTHUR WEIL and L. L. MAYER.

During experiments to produce retinal tumors by injecting carcinogenic chemicals into the eyes of white rats, the solvents lard and olive oil, alone, were found capable of stimulating the proliferation of retinal cells. Experiments were then made on white rats by injecting superheated lard into the vitreous. During the early stages a mass of granulation tissue developed. The ganglion cells at

this stage were increased in number and size. Three months after the injection of lard an increase in the width of the retina to two or three times its original width was observed, accompanied by an increase in the number and the size of the cells of the inner nuclear layer. At this stage the cytoarchitecture of the retina was preserved. Gradually the outer reticular layer was narrowed by the proliferation of the cells of both nuclear layers. Many rosettes were present; the small nuclei surrounding them had active mitosis. During the seventh month the newly formed tissue completely filled the chamber and invaded the optic nerve. The choroid was vascularized at this stage, but apparently there was no proliferation of the cells in the pigmented layer. The ciliary body was markedly hypertrophied.

DISCUSSION

PAUL A. STEINER: On what basis do you distinguish between neoplasia and hyperplasia? The characteristics of neoplasia include the penetration of the eyeball by the growth, the production of metastases and the ability of transplanted tissues to continue growth. Did the superheated lard become fluorescent and was the residual material saponifiable?

ARTHUR WEIL: In hyperplasia the normal structure of an organ is preserved, and the normal tissue cells are increased in number. Applying this definition, I may say that up to the fourth month following injection of lard into the corpus vitreum the retina shows hyperplasia. Later its cytoarchitecture is destroyed. At first the outer reticular layer is invaded by newly formed cells, but about the sixth month there is invasion of the inner reticular layer, which, with its dense layers of nerve fibers, forms a barrier hard to penetrate. Finally the entire posterior chamber is filled by the tumor. The optic nerve, too, is invaded. The retinal cells change their histologic appearance. While normally the nuclei of the inner nuclear layer measure from 5 to 6 microns and are hyperchromatic, the newly formed cells have large vesicular nuclei, 8 to 9 microns in diameter, and contain a few coarse granules of chromatin. If one adds to the proliferating growth the destruction of the normal structure, the invasion of the optic nerve and the active nuclear division observed during the sixth month, there should be no doubt that there is a neoplasia of the retina.

In human retinoblastoma, metastasis to other organs occurs rarely, but invasion of the optic nerve and finally of the brain is seen during the late stages. Fractionation of the lard was attempted. Separate fractions of the distillate up to 250 C. in vacuo were injected, without success. Saponification with potassium hydroxide destroyed the neoplastic properties. Trauma of the retina alone and injections of foreign bodies, such as a charcoal suspension, colloidal silver and cholesterol emulsions, did not lead to hyperplasia or tumor formation.

Enteric Intramural Herniation Following Colostomy. GEORGE J. RUKSTINAT, CHESTER B. THRIFT and FRANK M. SYLVESTER.

The factors predisposing to prolapse of the large bowel after colostomy are: the length of the mesentery, which permits intestinal evagination; the weakness of the abdominal wall as a result of improper technic or of atrophy as a sequel to suppuration, and altered innervation of the intestine. The direct cause of the prolapse may be active (e. g., a violent straining effort at stool) or passive (e. g., the aspiration action of a cup type of apparatus).

The anatomic forms of prolapse are: simple, involving only the mucosa of the colon; total, involving the entire wall of the colon; hernia of a small bowel into the colon; strangulated. This report concerns elements of all the foregoing types and, in addition, the complications of extensive herniation of the small bowel between the fascial layers of the abdominal wall and a fistulous passage from the urinary bladder to the distal part of the colostomy loop.

A white man aged 76 complained of scalding urine, looseness of the bowels and occasional drops of blood in the stools, which came from the proximal opening of his colostomy. Simultaneously with the passage of fecal matter from the

proximal colostomy opening, urine was discharged from the distal opening. Twenty-four years before the onset of these complaints he had not defecated for nine days and had had surgical intervention for a supposed tumor of the bowel. A pelvic mass, palpated at operation at that time, was not removed, but a permanent colostomy was established above the involved region. Thirteen years later a protruding portion of bowel was resected from the upper colostomy opening. Six years later diabetes mellitus developed, which was controlled by dietary treatment. On admission to the Loretto Hospital with the complaints stated, the patient had a pendulous abdomen from which the descending colon protruded 32.5 cm. from the proximal colostomy opening. When the bladder filled, urine passed from the distal opening. There was a large indurated mass in the pubic region, and another was palpated near the pylorus. Three weeks later the patient was critically ill and dehydrated. He had not defecated in forty-eight hours. He had cramping pain in the upper part of the abdomen, and a large firm mass occupied the left hypochondrium to the umbilicus. Death followed several days after ileocolic enterostomy.

The pertinent portions of the anatomic diagnosis were: hypostatic bronchopneumonia; primary carcinoma of the tail of the pancreas; generalized carcinoma metastases of the liver, peritoneum and biliary lymph glands; marked extrusion of the proximal colostomy loop, and herniation of the small bowel intramurally into the abdominal wall about the colostomy and into the protruding colostomy loop; vesicocolic fistula. The lumen of the distal portion of the colon and rectum was only 3 to 5 mm. in diameter; the wall was 8 mm. thick, indurated and pale gray-white. The fistulous tract was surrounded by connective tissue and small nests of lymphocytes. The absence of carcinoma from this part of the bowel suggests that the condition for which the original colostomy was done was probably a diverticulum of the colon. Inflammation of the wall of the diverticulum led to obstructions of the bowel, adhesions between the bowel and the bladder and, eventually, perforation of the bladder.

Hypertension (Goldblatt) and Unilateral Malignant Nephrosclerosis. Otto Saphir.

Two patients had severe arterial hypertension secondary to unilateral renal vascular stenosis with consequent ischemia of one kidney. Both of these had at autopsy unilateral malignant nephrosclerosis. This unique finding could be explained by recent experimental evidence. Goldblatt concluded from his experimental studies, primarily concerned with the production of arterial hypertension by clamping the renal arteries in dogs, that both hypertension and renal insufficiency are the minimal prerequisites for the induction of arteriolonecrosis, for in the absence of either of these factors no necrotizing changes are observed. In each case reported here severe arterial hypertension was brought about by renal arterial changes resulting in ischemia of one kidney. Renal excretory insufficiency subsequently developed in both cases. It was precipitated in one instance by the onset of congestive heart failure and in the other by the development of acute ascending pyelonephritis in the kidney opposite the ischemic one. Because of the presence of the severe arterial hypertension and excretory renal insufficiency, the arterioles in the contralateral kidneys, with patent vascular system, showed necrotic changes, and these kidneys presented the typical picture of malignant nephrosclerosis. The arterioles in the ischemic kidneys revealed no necrotic changes because the stenosis of the renal and intrarenal arteries militated against the presence of severe hypertension within the arterioles. Thus, the genesis of the malignant nephrosclerosis in these cases is exactly similar to that of the arteriolonecrotic changes produced experimentally by Goldblatt.

Dystrophic Calcification of the Myocardium with Glomerulonephritis. R. M. Bolman.

This article will appear in full in a later issue of the Archives.

Book Reviews

Virus and Rickettsial Diseases, with Especial Consideration of Their Public Health Significance. A Symposium held at the Harvard School of Public Health, June 12-17, 1939. Cloth. Pp. 907, illustrated. Price, $6.50. Cambridge, Mass.: Harvard University Press, 1940.

After twenty years of intensive investigation the world over, there has accumulated such a wealth of detailed knowledge concerning the filtrable viruses and the diseases they cause that need has arisen for a comprehensive and critical orientation of the subject matter and theory both for the investigator himself and for students of medicine and allied sciences. In response to this want, several excellent treatises have appeared in recent months, prepared by individual authors or by groups of authors, themselves authorities within their special fields. The symposium held at the Harvard School of Public Health in June 1939 represented a response to the aforementioned need for a discussion and general consideration of virus diseases especially from the standpoint of public health, and the volume under review is the published product of that conference. A general survey of rickettsial diseases, their clinical features, diagnosis, classification, epidemiologic features and immunity, was included because of certain analogies obtaining between them and the filtrable virus groups that have caused some confusion.

It is significant of the present absorbing interest in the virus field that most of the twenty-four authors of the thirty-four papers comprising the volume are investigators actively engaged in research on certain phases of the subject they present, and at a single university or in its environment.

Each paper is a review of important advances in knowledge and theory related to the subject matter and is followed by a reasonably full bibliography. The rather comprehensive introductory paper deals with "Epidemiologic Problems in Virus Diseases" and serves both to introduce the book and to emphasize the public health aspects of the diseases under consideration. This is followed by an adequate consideration in three papers of physical and chemical properties, immunologic aspects and insect vectors of viruses and their diseases.

Subsequent papers deal almost exclusively with the more important virus diseases of man, namely, variola-vaccinia, measles, mumps, dengue fever, venereal lymphogranuloma, influenza, psittacosis, poliomyelitis, rabies, equine encephalomyelitis, lymphocytic choriomeningitis, louping ill and yellow fever, in the order mentioned.

The several contributions are for the most part of excellent critical quality, though a few are rather summary. Certain contributors are, as might be expected, more at home when dealing with special phases of individual problems than in a general consideration of theory and outlook. The subject of viruses is perhaps not yet quite ripe for indulgence in generalization, but there is noticeable a certain haziness of ideas as well as looseness in the use of terms that even now should be applied more precisely. It seems to this reviewer, for example, no longer justifiable to use the term "ultramicroscopic viruses" with comprehensive significance when it is well known that the elementary bodies of such virus lesions as those of fowlpox, molluscum contagiosum and others can be very easily seen in fresh preparations through a high power oil immersion system and that these structures might well be the formal representations of the respective active agents so far as observers know at present. Furthermore, while the authors generally accept the point of view that viruses invade and undergo multiplication inside of cells as necessary conditions to infection, there seems at times to be a lack of critical appreciation of the desirability of more precise knowledge respecting this cytotropic property, especially from the standpoint of clarity in thinking and in

investigation of pathogenesis. For example, in the discussion of classification of virus diseases of the central nervous system (p. 617) some point is made to criticize a rather loose use of the adjective "neurotropic," yet the author himself indulges with unconscious ease and evident innocence in the acceptance and use of such indefinite and confusing adjectives as "pantropic" and "viscerotropic" as bases for orderly arrangement. It is refreshing to see one author (p. 720) take exception to such confusion, although nowhere in the book is the basic hypothesis of cytotropism of viruses clearly and understandingly considered.

The volume as a whole is a timely one and will be of great help to students, teachers and investigators of disease generally.

Diseases of the Gallbladder and Bile Ducts. Waltman Walters, B.S., M.D., M.S., Sc.D., and Albert M. Snell, B.S., M.D., M.S. Pp. 645. Price $10. Philadelphia: W. B. Saunders Company, 1940.

One of the important advances in modern medicine has been the development of reviews of subject material in monograph form. The reviews in the basal sciences have for the most part been characterized by a greater depth of scholarship than is apparent in those in the clinical fields of medicine. This book by Walters and Snell, with eight of their associates at the Mayo Clinic, can be compared favorably with the best of the reviews. While little appears that is new, the authors have gathered together a great deal of published data bearing on the biliary tract from many angles, and the compilation is equal to the high standards which one has come to associate with the work of the Rochester group. The bibliography is superb and is a good point of reference for wider studies.

The anatomy, physiology and pathology of the biliary tract are reviewed, and then the clinical aspects of diseases in this system are considered, followed by details of medical and surgical treatment. The surgical aspects of the field are considered in the greatest detail. The book is well illustrated with 195 figures.

In parts the book is repetitious, and the etiology of gallstones and the pathology of cholecystitis are discussed in several scattered places. A second edition would profit by more efficient arrangement and condensation. Several contradictory statements are made at times by the same author. Thus, one reads: "A review of this large series of surgically removed gallbladders suggests that gallstones, regardless of whether they are metabolic or bacterial in origin, constitute the greatest menace to the welfare of their hosts" (p. 86), and "Judging from the fact that according to postmortem records a relatively high percentage of individuals have stones and the fact that so very few people die directly from gallstones in the gallbladder it might be suggested from this material that removal of gallstones simply because they are found roentgenoscopically is not justified, especially if their host has no incapacitating signs and symptoms." (p. 87.)

The chemical nomenclature at times is loose; thus, sodium hypochlorite is referred to as "chlorinated soda" (pp. 45 and 47), and such statements as "white stones are largely composed of calcium" (p. 84) are obviously rich in misnomers. One also could wish for a treatment of precipitation in bile from the standpoint of physical chemistry.

The book is highly recommended to those especially interested in the biliary tract.